Quantifying Corn Deterioration Due to Fungal Growth by Use of CO_2-Sensitive Gel

S. Chitrakar, C. J. Bern, D. S. Shrestha

Abstract. *Carbon dioxide (CO_2) generation is a useful measure of aerobic respiration of the microbes that decompose organic materials. Woods End Labs markets the Solvita® test kit to measure CO_2 generated by samples of compost or soil. A procedure was developed to use the Solvita® kit to quantify the storage condition of shelled corn. The ISU-Solvita® Corn Testing Procedure uses shelled corn stored at 20°C. After a 24-h incubation period, a CO_2-sensitive gel coated paddle is sealed in a jar containing 100 g of corn. After 4 h, the CO_2 level in the jar is indicated by the color of the gel. The ISU-Solvita® Corn Testing Procedure was shown to be capable of quantifying the storage state of corn over a range of moistures and durations of incubation after re-wetting. A linear relation was observed between corn moisture and measured %CO_2 for moistures between 18% and 22%. An exponential relation was observed between measured %CO_2 and storage times of 20% moisture corn from 3 to 12 days at 27°C. In other tests in which samples were rewetted to 20% moisture and incubated 24 h, there was no relationship between corn with visible mechanical damage or corn bulk density and measured CO_2.*

Keywords. *Corn storability, Corn deterioration, Carbon dioxide evolution.*

After wheat and rice, corn (*Zea mays* L.) is the third most important grain crop in the world with production of nearly 602 Gg in the year 2002, of which about 40% was produced in the United States (FAO, 2003). Corn is grown in nearly all countries with suitable climatic conditions and harvested at moistures between 30% and 15% (all moistures are % wet basis). After harvest, the stored corn is subject to deterioration over time due to growth of fungi that can damage or destroy the kernels, and consequently diminish the corn's commercial value. Appropriate storage conditions are needed in order to preserve corn quality until final use. It is also important to be able to track the level of deterioration of stored corn through its storage life.

Measuring Deterioration

All aerobic organisms, including viable grain, respire and in this process dry matter is oxidized and CO_2 is evolved. Respiration of a stored corn mass can be classified into respiration of viable corn kernels and respiration of various organisms that live on the kernels. The contribution of the viable corn kernels is usually assumed to be negligible. However, Seitz et al. (1982) observed rapid dry matter loss from fungus-free corn in a laboratory experiment. CO_2 evolution is commonly used as an index of respiration of a corn mass. It has been widely used in tracking deterioration of shelled corn during storage (Saul and Steele, 1966; Steele, 1967; Steele et al., 1969; Fernandez et al., 1985; Friday et al., 1989; Al-Yahya et al., 1993; Wilcke et al., 1993; Aljinovic et al., 1995; Dugba et al., 1996; Ng et al., 1998). The deterioration process has been modeled by Saul and Steele (1966) and others as the complete oxidation of glucose:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2835\ \text{kJ/mole} \quad (1)$$

According to this equation, a 1% loss from 1 kg of dry matter (10 g of glucose) is accompanied by the evolution of 14.7 g of CO_2. On average, corn can lose 0.5% of its dry matter (7.35 g of CO_2 per kg of dry matter) through deterioration before quality is reduced by one USDA grade (Saul and Steele, 1966). Therefore, by measuring the CO_2 production over time at a given moisture content and temperature, the extent of deterioration can be quantified as the loss in dry matter. The following equation can be used to predict CO_2 production from corn (Bern et al., 2002):

$$Y = 1.3\,(e^{0.006\,t/m} - 1) + 0.015\,t/m \qquad (2)$$

where

Y = grams of CO_2 produced per kg of original corn dry matter

t = time (h)

m = $M_M M_T M_D M_H M_F$

M_M, M_T, M_D M_H, and M_F are dimensionless multipliers used to account for corn moisture content, temperature, mechanical damage level, genetic hybrid resistance and fungicide treatments, respectively. Equations for calculating multipliers are defined in Bern et al. (2002).

Measuring CO_2 Produced

Various techniques have been used by researchers to measure CO_2 evolution from stored grain. The most common techniques involve capturing and weighing CO_2 present in aeration air (Steele, 1967; Steele et al., 1969; Fernandez

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The authors are **Sinora Chitrakar,** former Graduate Student, School of Environment Resources and Development, Asian Institute of Technology, Bangkok, Thailand; **Carl J. Bern, ASABE Fellow Engineer,** University Professor, Department of Agricultural and Biosystems Engineering, Iowa State University, Ames Iowa; and **Dev S. Shrestha, ASABE Member Engineer,** Assistant Professor, Department of Biological and Agricultural Engineering, University of Idaho, Warsaw, Idaho. **Corresponding author:** Carl J. Bern, Iowa State University, 217 Davidson Hall, Ames IA 50011; phone: 515-294-1270; fax 515-294-2552; e-mail: cjbern@iastate.edu.

et al., 1985; Al-Yahya et al., 1993; Dugba et al., 1996) or detecting CO_2 in aeration air (Wilcke et al., 1993; Ng et al., 1998).

These methods have proven to be effective in tracking corn deterioration over time. However, they are time consuming and require specialized equipment and knowledge. A simpler, faster method of measuring CO_2 evolution during corn storage could be a valuable tool for assessment of deterioration caused by growth of storage fungi.

SOLVITA® KIT

Woods End Labs developed and markets the Solvita® test kit to measure CO_2 generated by a small quantity of compost or soil (Woods End Research, 2002). Each kit consists of a 237-mL (half pint) plastic jar, a color scale, and a packet containing a small polystyrene paddle that has a CO_2 sensitive gel surface on one side (fig. 1). The gel contains a pH buffer and changes color according to the CO_2 concentration in the jar. CO_2 inside the bottle diffuses into the gel, neutralizes the buffer causing a pH drop that in turn induces the change in gel color. The color number is determined by comparing the color of the gel with the colors on a color card that has numbers assigned to a series of colors that represent the progression of color change as the CO_2 concentration in the jar increases. Only a small fraction of the CO_2 in the jar is absorbed by the gel at equilibrium, but the color change is proportional to the CO_2 concentration in the air inside the jar. The relationship of color number and CO_2 concentration for the Soil Kit packet is provided by Woods End Laboratory (table 1, %CO_2, Woods End). According to Woods End, CO_2 concentrations greater than what is indicated by color 5 will produce a color not on the card.

Seekins (1996) tested this kit for compost maturity determination and concluded that the test was a useful tool as long as limitations such as prepartion of the sample, the time taken for the color change and the identification of the color change were kept in mind. McDonnell and Regenstein (1997) evaluated the Solvita® kit to determine its usefulness when used by untrained personnel working with compost at a variety of sites lacking the equipment and environmental control of a laboratory. They found that the test was an accurate, quick and convenient means of assessing the activity, or stability, of a given compost. The reviewers found that the kit was simple to use and the results were easy to interpret.

Figure 1. Solvita® test kit: plastic jar, color reading card, Solvita® packet, Solvita® paddle, and color card.

Table 1. CO_2 concentration corresponding to Solvita® color numbers.

	Solvita® Color No.							
	0	1	2	2.5	3	3.5	4	5
%CO_2, Woods End	< 0.1	0.2	0.4	0.6	0.8	1.1	1.5	3.0
%CO_2, ISU	−−	0.0	0.2	0.4	0.6	1.0	1.4	3.1

Brewer and Sullivan (2003) used Solvita® kits to measure respiration rates of yard trimmings compost. Grab samples for testing were drawn from compost windrows 11 times over 133 days of composting. Tests were performed at 18°C, and paddle color numbers were read after 4 h. Using the Solvita® results they were able to identify a period of high respiration rates (first 27 days) and a period of low and stable respiration rates (between 70 and 133 days). They were also able to use the test results to distinguish between composts which had been aerated with fans, and unaerated compost.

Changa et al. (2003) used Solvita® kits in their study of composted swine and dairy manure, mixed with sawdust or ground wood pallets. Samples said to be at 50% to 55% moisture were periodically drawn from outdoor windrows over three months and then stored overnight at 25°C. Paddle color numbers were read after 4 h. When successive samples were removed from the windows over a period of several months, CO_2 values for all three composts decreased over time. They followed similar trends and reached low, relatively constant values after 84 to 98 days, signifying that the compost had been stabilized. Solvita® CO_2 values were highly correlated with respirometer CO_2 values for all three.

The Solvita® kit is also used to test soil quality. Measurement of soil CO_2 evolved as a result of microbial respiration is a way of gaging biological activity of living microorganisms in the soil (Doran et al., 1997). Doran et al. (1997) evaluated the Solvita® soil test kit and concluded that “the Solvita® system offers great promise as a substitute for more refined methods of quantitatively measuring soil respiration both in the laboratory and the field.”

Motivated by the successul use of Solvita® kits in measuring CO_2 from compost and soil, we saw value in evaluating its use with shelled corn. The objective of this study was to develop a procedure for conducting tests on shelled corn using the Solvita® CO_2 measurement system and then to use this procedure for quantifying the effects of corn moisture content, time of storage, level of visible mechanical damage, and bulk density on the level of deterioration of shelled corn during storage.

METHODOLOGY

The extent of fungal growth on corn samples was determined by measuring CO_2 evolved from the samples. CO_2 production was quantified by observing the color of gel on the Solvita® paddles in sealed glass jars containing corn samples re-wetted to specific moistures and kept at 20°C.

Tests were conducted in the Biomaterials Lab in Davidson Hall at Iowa State University.

CALIBRATION OF THE CO_2-SENSITIVE GEL

The relationship between the color of the gel on the paddles and the CO_2 concentration in the surrounding atmosphere was determined and compared to data provided by Woods End. Calibration was carried out using an AC'SCENT™ International olfactometer (St. Croix Sensory Inc., Stillwater, Minn.) located in Davidson Hall. The olfactometer was used only to provide precise CO_2 concentrations in plastic bags. Details of the calibration are described in Chitrakar (2002). CO_2 concentrations of 0.195%, 0.39%, 0.78%, 1.56%, and 3.15% were used for this calibration. Solvita® paddles were inserted into airtight plastic bags containing each CO_2-air mixture. The Solvita® color number of the Solvita® paddle was noted when paddle color no longer changed. Since each bag contained at least 5 L of gas, we assumed that insertion of a paddle did not significantly alter the CO_2 level of the bag contents. A graph of %CO_2-ISU calibration versus %CO_2-Woods End calibration was drawn and a linear model was fitted using Microsoft Excel (fig. 2). ISU calibration concentrations for each Solvita® color number were calculated (table 1). ISU calibration concentrations were used for all tests reported in this article.

SAMPLE PREPARATION

Dry corn lots of each hybrid tested were stored at 5°C until use and then were rewetted to desired moisture levels by adding distilled water from a spray bottle and storing in polyethylene bags at about 3°C for at least 24 h. According to Fernandez at el. (1985), an acceptable method of storing corn for storage experiments is drying and rewetting. They found that drying corn to 14.7% moisture and then rewetting did not significantly change CO_2 production compared to that of freshly harvested corn. Moisture content was determined after the CO_2 experiment.

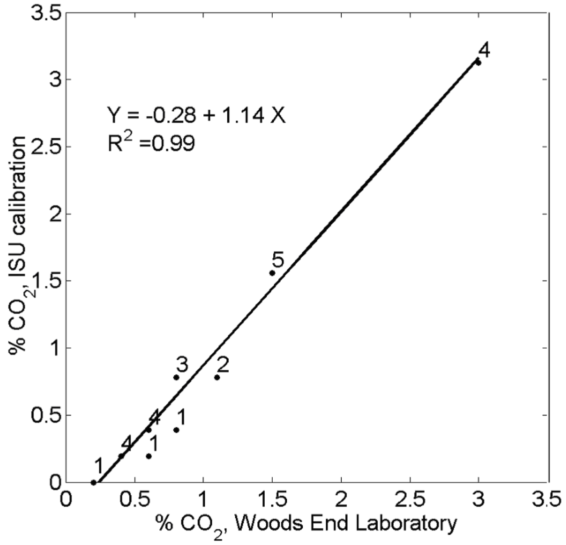

Figure 2. ISU calibration vs. Woods End Laboratory %CO_2. Figures above points indicate number of data points represented.

ISU-SOLVITA® CORN TESTING PROCEDURE

Tests were conducted by placing 100-g corn samples in airtight Kerr 473-mL (1-pint) glass jars (Alltrista Corporation, Munice, Ind.) previously autoclaved at 120°C for 30 min. Glass jars were used instead of plastic jars that came with the kit (fig. 1) because paddle colors are easier to read through glass (Stroshine 2000). Jars containing corn samples were placed in a controlled temperature chamber in which a fan was continuously circulating air (Model I-35LLVL, Percival Scientific Inc., Perry, Iowa) at 20° ± 0.1°C along with empty jars for 24 h so that all jars and samples were at a constant temperature at the beginning of the test. During this incubation period fungal activity will increase and the rate of CO_2 production will increase in proportion to the rate of fungal growth. Therefore, at the end of the incubation period there will be more CO_2 produced during the 4-h Solvita® test period. Woods End recommends using Solvita® paddles at temperatures between 20°C and 25°C (Woods End Research, 2002). After 24 hours, corn samples were poured into empty jars inside the controlled temperature chamber, a Solvita® paddle was inserted into each bottle, and its lid was screwed on tightly. Solvita® color numbers were read four hours after the paddles had been inserted and the values were converted to percent CO_2 using the ISU calibration (table 1). Preliminary testing showed that commodity corn at moistures around 20% usually produced CO_2 concentrations within the Solvita® ISU calibration range when tested at 20°C using this procedure. Following determination of the color number, the entire sample was placed in a metal can for moisture determination. The moisture content was determined by placing the cans in an oven at 103°C for 72 h (*ASAE Standards*, 2003). This procedure was used for all four experiments.

DETERIORATION MEASUREMENTS

Experiment 1: Effect of Corn Moisture on CO_2 Produced

Experiment 1 was conducted to measure CO_2 released by corn samples at different moisture contents. Corn used in this study was Wilson hybrid 1664 obtained from Wilson Genetics, LLC, Harlan, Iowa. This corn was hand-picked, dried on the ear with 32°C air, and shelled in a motorized laboratory sheller. A 70-kg lot of corn at 12.7% moisture was cleaned by using a 4.8-mm (12/64-in.) round-hole sieve and then stored at 5°C until use. Prior to testing, samples of corn were rewetted to target moistures of 18%, 19%, 20%, and 21%. Five replicates of subsamples of each of these corn samples were tested in random order using the ISU-Solvita® Corn Testing Procedure.

Experiment 2: Effect of Storage Time on CO_2 Produced

Tests were conducted on Wilson hybrid 1664 corn samples incubated for various time periods after rewetting to see if the Solvita® test is capable of determining the deterioration of the corn caused by fungal growth. This corn was from the same 12.7% moisture, 70-kg lot used for Experiment 1. Five 1000-g corn samples were first wetted to 20% moisture and kept at 5°C for 24 h. The samples were placed in an incubator at 27°C for 3, 6, 9, or 12 days and then stored at 5°C until the Solvita® tests were carried out. After all five samples had been incubated, five subsamples from each sample were tested using the ISU-Solvita® Corn Testing Procedure. CO_2 concentrations obtained from this experiment were used to calculate the grams of CO_2 produced per gram of corn dry matter. These values were compared to the CO_2 production calculated using equation 2 from Bern et al. (2002).

Experiment 3: Effect of Visible Mechanical Damage Level on CO_2 Produced

Solvita® tests were conducted on corn samples having various visible mechanical damage levels. The corn hybrid used in this experiment was Garst 8543 IT, combine harvested at 12% moisture. The combine harvester was adjusted to inflict heavy mechanical damage. Kernels were sorted manually by visual inspection into damaged and undamaged categories, and then stored at 5°C until they were used for the Solvita® tests. Mechanical damage was defined as visual ruptures or breaks in the seed coat (Schmidt et al., 1968; Steele, 1967). The sorted corn was mixed by combining appropriate weights of damaged and undamaged kernels for 0%, 25%, 50%, 75%, and 100% (by weight) damage levels. These samples were then rewetted to 20% moisture and tested in random order using the ISU-Solvita® Corn Testing Procedure.

Experiment 4: Effect of Bulk Density on CO_2 Produced

Corn samples from the experiments of Krueger (2002) were used in this experiment. Commodity corn of unknown hybrid at 13.5% moisture was purchased at the West Central Coop, Boone, Iowa elevator and separated using a gravity table into fractional bulk densities of 671, 708, 728, 743, and 751 kg/m^3. Bulk densities were measured using a standard USDA corn test weight procedure. Samples were re-wetted by adding the calculated amount of water needed to increase the moisture content to 20%. Three sub-samples from each sample were tested using the ISU-Solvita® Corn Testing Procedure.

Results and Discussion

Experiment 1: Effect of Corn Moisture on CO_2 Produced

Measured $\%CO_2$ was plotted against corn moisture content and a linear model was fitted (fig. 3). Moisture contents plotted were determined by oven test following the CO_2 experiment. The model had a coefficient of determination, R^2, of 0.93. This indicates there was a linear relationship between the 4-h CO_2 accumulation in the jar and corn moisture content. The slope of the line (0.26 percentage points of CO_2 per percentage point of moisture) shows the test's high sensitivity to grain moisture, and indicates that grain moisture must be closely controlled or accounted for when using Solvita® tests.

The percent CO_2 indicated by Solvita® color was converted into grams of CO_2 per kg of corn dry matter using 1.83g/L as the density of CO_2 and the air volume inside the jar computed as 413 cm^3. Results of these tests were compared (fig. 4) to the calculated (eq. 2) mass of CO_2 produced by corn between its 24th and 28th hours of incubation at 20% moisture and 20°C. In equation 2, $M_D = 1.64$ assuming $D = 10\%$, $M_T = 0.642$, $M_M = 2.95$, $M_H = 1$, $M_F = 1$.

Experiment 2: Effect of Storage Time on CO_2 Produced

Figure 5 shows storage time versus log $\%CO_2$ for corn incubated at 27°C and 20% moisture for 3 to 12 days. Moistures determined after testing ranged from 19.1 to 22.4, and averaged 20.9%. An exponential model was fitted to the data. With a model R^2 over 0.8, the results show that the ISU-Solvita® Corn Testing Procedure is able to discern the storage condition or deterioration state of shelled corn. The procedure has the potential to predict future deterioration of

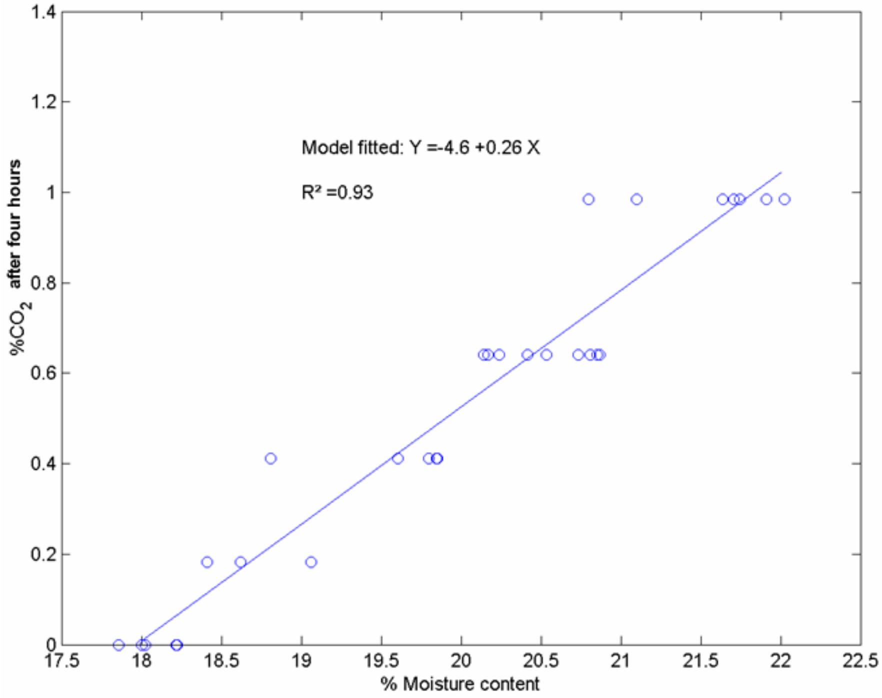

Figure 3. Relationship between corn moisture content and the percent CO_2 in glass sample jar 4 h after the sample was placed in the jar. Tests were conducted at 20°C. Each point represents one reading.

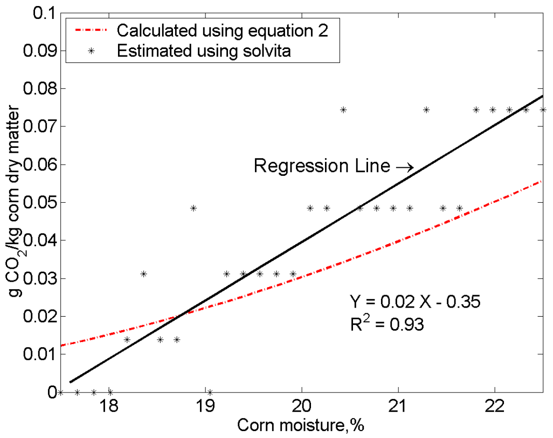

Figure 4. Measured and predicted percent carbon dioxide in the air in glass jars 4 h after samples at various moisture contents were placed in the jars. Tests were conducted at 20°C. Each point represents one reading.

stored corn. More research is needed to refine the procedure. The upper line of figure 5 was calculated using equation 2. CO_2 was calculated assuming corn was incubated at 27°C for 3, 6, 9, or 12 days, then for 24 h at 20°C. Then the CO_2 collected in the jar during 4 h of incubation at 20°C was calculated. There is reasonable agreement between the calculated CO_2 quantity and the measured CO_2 quantity.

EXPERIMENT 3: EFFECT OF VISIBLE MECHANICAL DAMAGE LEVEL ON CO_2 PRODUCED

The observed relationship between %CO_2 produced and % visible mechanical damage is plotted in figure 6. Moistures determined after testing ranged from 18.9 to 23.4 and averaged 21.0%. ANOVA analysis showed that CO_2 evolution rate was not significantly different (F = 0.61; P > F = 0.67) for damage ranging from 0% to 100%. This result does not agree with the previous research which has found that corn CO_2 production (and corn deterioration rate) increases sharply as % visible mechanical damage increases. The M_D (damage) multiplier equation in Bern et al. 2002 predicts that the CO_2 production rate will increase by a factor of 2.5 as visible mechanical damage increases from 3% to 41%. A likely explanation for this discrepancy is the short incubation time of these samples. The 4-h test took place after an incubation period of 24 h. It is likely that the effects of damage would be more evident if the corn had been incubated for a longer time prior to the CO_2 evolution test.

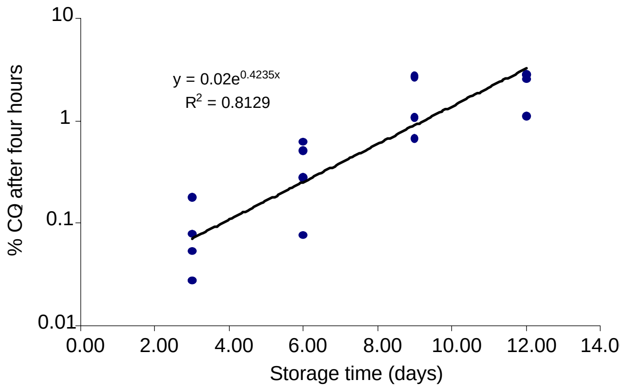

Figure 5. Percent CO_2 in the air in glass jars 4 h after samples were placed in the jars. The sample had been -wetted to 20% moisture content and incubated for 3 to 12 days at 27°C. CO_2 kit tests were conducted at 20°C. Each point represents one reading.

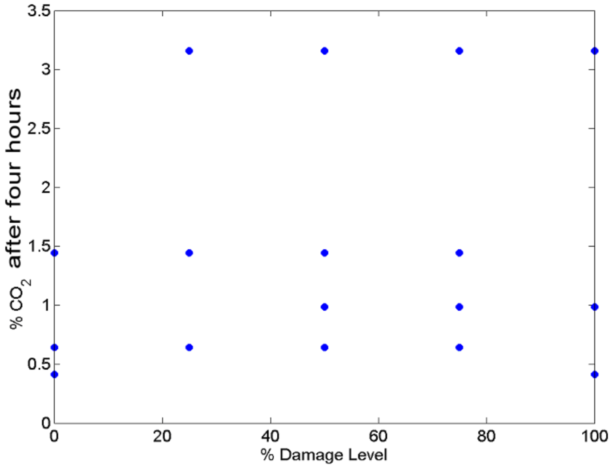

Figure 6. Percent CO_2 in the air in glass jars 4 h after samples having different levels of mechanical damage were placed in the jars. After the samples were re-wetted to 20% moisture content, their temperature was maintained at 20°C. Each point represents one reading.

EXPERIMENT 4: EFFECT OF BULK DENSITY ON CO_2 PRODUCED

The observed relationship between %CO_2 produced and corn bulk density is plotted in figure 7. A one-way ANOVA showed that there was no significant difference in CO_2 production between any of the bulk densities. (F = 0.93; P > F = 0.48). There is a general belief in the grain industry that low test weight corn deteriorates more rapidly during storage, compared to higher test weight corn. However, corn storage experiments supporting this belief have not been recorded in the literature.

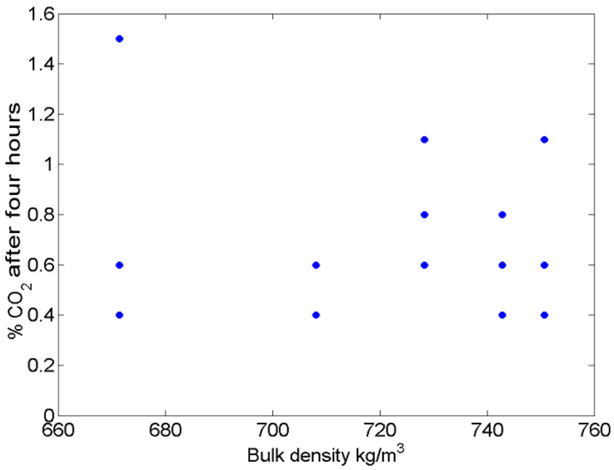

Figure 7. Percent CO_2 in the air in glass jars 4 h after samples having different bulk densities were placed in the jars. After the samples had been re-wetted to 20% moisture content, their temperature was maintained at 20°C. Each point represents one reading.

FUTURE RESEARCH

Additional testing using longer incubation times is appropriate to detect probable effects of visible mechanical damage and corn bulk density on CO_2 evolution. As suggested by a reviewer, use of hand-shelled kernels rather than combine-harvested kernels with no visible mechanical damage might improve the likelihood of detecting effects of mechanical damage of kernels. Germination testing of samples could determine the contribution of corn respiration to the CO_2 evolved, to ensure it would not mask effects of fungal growth.

SUMMARY AND CONCLUSIONS

The Solvita®, kit was evaluated as a means of measuring the concentration of CO_2 produced by corn samples at various moisture contents, duration of incubations after re-wetting, damage levels, and bulk densities. The ISU-Solvita® Corn Testing Procedure was developed and then used for these measurements. The following results indicate that the testing procedure was able to quantify effects of moisture content and incubation time on fungal growth in shelled corn:

- A linear model ($R^2 = 0.93$) described the relationship between corn moisture content and % CO_2 measured, for moistures between 18% and 22%.
- An exponential model ($R^2 = 0.81$) described the relationship between corn storage time at 20% moisture and 27°C, and % CO_2 for storage times from 3 to 12 days.
- In other tests, in which samples were re-wetted to 20% moisture and incubated for 24 h, there was no relationship between measured CO_2 and either visible mechanical damage or corn bulk density.

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