THE CRITICAL MOISTURE RANGE FOR RAPID MICROBIAL DECOMPOSITION OF RICE STRAW DURING STORAGE

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ABSTRACT. *Proper design of biomass storage systems is a critical factor facing lignocellulose-based industries. Improper storage conditions can result in decomposition and deterioration of biomass, and self-heating to the point of ignition. The goal of this research was to characterize microbial activity on a model lignocellulosic resource, rice straw, between 20% and 200% moisture content on a dry basis (d.b.) at 25˚C and 35˚C. Microbial activity was determined from CO_2 evolution rate measurements made on batch incubations of wetted straw. The threshold moisture content below which microbial activity would not occur was found to be between 29% and 41% d.b. Microbial activity, represented as peak and cumulative carbon dioxide evolved, followed a sigmoidal relationship with respect to moisture content; activity increased exponentially with moisture contents between 30% and 100% d.b. and then leveled off between 100% and 250% d.b. The rate at which the biomass achieved peak activity increased with increasing moisture. Activity and the rate of self-heating were greater at 35˚C compared to 25˚C and suggest a greater risk for biomass deterioration, self-heating, and thermal runaway conditions under higher mesophilic temperatures.*

Keywords. *Biological activity, Moisture content, Respiration, Rice straw, Self-heating.*

Successful use of plant residues or dedicated crops for lignocellulose-based industries requires a reliable and consistent supply of high-quality materials (CARB, 2001; Dobie et al., 1977; Kadam et al., 2000). Since crop harvest generally occurs on a seasonal basis and often when conditions are wet, harvested biomass must be stabilized appropriately and stockpiled for several months to create a consistent year-round supply (Dobie et al., 1977). One stabilization approach appropriate for warm and dry climates is to dry the material prior to harvest. While microbial activity is not expected in properly field-dried and baled biomass, the introduction of moisture due to a rainfall event or flood would stimulate microbial activity. Therefore, it is important to characterize the response of microorganisms to various levels of available moisture to properly select storage design alternatives and evaluate their risks to decomposition and thermal runaway.

Availability of water is a critical factor for microorganisms to function (Madigan et al., 2000); lack of available water on solid substrates is a significant source of microbial stress (Griffin, 1981; Richard et al., 2002). Microorganisms need water for motility and transportation of nutrients to cells and waste products away from cells (Gervais et al., 1996; Madigan et al., 2000). Native microbial activity in hay and other agricultural residues has been studied extensively in the context of self-heating to the point of spontaneous combustion. Many of these studies have noted the importance of available moisture. For example, in hay, little or no microbial activity is expected below 25% to 30% on a wet basis (w.b.) (Bowes, 1984; Festenstein et al., 1965; Rothbaum, 1963). Higher moisture contents should be conducive to more microbial activity because more bacteria would be able to thrive. In hay that was allowed to self-heat, the maximum temperature of a bale increased with increasing initial moisture content (Rothbaum, 1963). The increased ability to self-heat hay was attributed to higher levels of microbial activity.

The objective of this study was to examine the effect of moisture on the microbial activity on rice straw, a model source of lignocellulosic biomass, during storage. Conditions in a rice straw bale range between anaerobic and aerobic; however, higher levels of microbial activity were expected under aerobic conditions. Therefore, all experiments were conducted at ambient oxygen levels. Rice straw moisture contents at the time of harvest vary between 40% and 75% w.b., and it is typically field-dried to 18% w.b. or below before baling (Dobie et al., 1977; Thompson, 1974). Although rice straw is a low-density biomass, the high yield of straw per acre gives it great potential as a lignocellulosic biomass resource (Kadam et al., 2000). Applications for harvested rice straw include ruminant feed, fiber and construction products, pulp and paper, adsorbents, and the production of energy via combustion, gasification, anaerobic digestion, and fermentation to ethanol (Juliano, 1985; Kadam et al., 2000; Marshall, 2004). Here, moisture levels that pose a risk for biomass deterioration and self-heating were identified.

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MATERIALS AND METHODS

RICE STRAW PREPARATION

The rice straw was a medium-grain Japonica (variety M-204) that was field-dried to 13% on a dry basis, collected

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and baled immediately after grain harvest in Yolo County, California, in November 2001 (Summers, 2005). The bale was used in a long-term storage study, where it was stored in a fully enclosed warehouse that protected the bale from moisture and wind but was not otherwise environmentally controlled. During the long-term storage study, solids and proximate analysis data did not change significantly, and internal bale temperature variations corresponded to fluctuations in ambient temperature (Blunk et al., 2003). Approximately 18 months after collection, rice straw from the bale was cut with a band saw into 25 mm to 100 mm pieces to allow it to be compressed into 250 mL bioreactors.

Initial moisture content (MC) of rice straw was 11% on a dry basis. Target moisture contents for experiments were 20%, 25%, 30%, 35%, 50%, 100%, and 200% on a dry basis. To achieve target moisture contents and uniform wetting for experiments, rice straw samples were placed in 9.5 L zipper plastic bags, sprayed with a fine mist of distilled water by weight, and mixed in the bags. The rice straw samples were allowed to equilibrate at 4°C for 20 h before filling reactors.

Rice Straw Incubation

Rice straw was incubated in bioreactors made from 250 mL HDPE containers (Nalge Nunc International, Rochester, N.Y.) with Teflon luer lock fittings, o-rings, and securing nuts at the bottom of the container for the inlet and in the lid for the outlet. Rice straw was supported on stainless steel mesh screen with 2.5 cm screws for legs. Trimmed aluminum drain guards were used to compress wetted rice straw into reactors when the lids were closed. All reactor parts were autoclaved before and after use. To simulate the density of a bale, 20 dry grams of rice straw were compressed into 220 mL working volume, for a density of approximately 90 kg m^{-3}.

Reactors were maintained at 25°C or 35°C in incubators. Clear, autoclavable PVC tubing with 3.2 mm and 6.4 mm inner diameters (Nalge Nunc International, Rochester, N.Y.) were used throughout the system. House compressed air was humidified by bubbling it through distilled water and metered to each bioreactor with polycarbonate rotameters (5 to 50 mL air min^{-1}, Dwyer Instruments, Inc., Michigan City, Ind.). A constant flow of 30 mL min^{-1} was supplied to each reactor. Water was removed from the effluent of each reactor by passing through molecular sieves (3A, beads, 8 to 12 mesh particle size, Sigma-Aldrich, St. Louis, Mo.) mixed with a small amount of indicating Drierite (W. A. Hammond Drierite Co., Ltd., Xenia, Ohio).

Dry effluent from reactors was plumbed to a 16-position switching valve (VICI Valco Instruments, Houston, Tex.), which switched positions every 20 min as controlled by a personal computer running Lab Tech Notebook software (CambridgeSoft Corp., Cambridge, Mass.). The effluent from the current position of the valve was sent to a carbon dioxide (CO_2) sensor (GMT221, Vaisala, Woburn, Mass.).

Carbon dioxide evolution rate (CER) has been correlated to growth of microorganisms in solid-state fermentations (Sato and Yoshizawa, 1988); therefore, it was used as a measure of microbial activity in this study. CER in mg CO_2 g^{-1} dry straw day^{-1} was calculated from outlet and inlet CO_2 data as follows:

$$\mathrm{CER} = F\left(\mathrm{CO}_{2,out} - \mathrm{CO}_{2,in}\right) \quad (1)$$

where $CO_{2,\,out}$ is the concentration of carbon dioxide in the effluent stream (mg CO_2 mg^{-1} air), $CO_{2,\,in}$ is the concentration of carbon dioxide in the influent stream (mg CO_2 mg^{-1} air), and F is the flow rate (mg air g^{-1} dry straw day^{-1}).

Sample Analysis

Moisture content for rice straw samples was obtained gravimetrically by drying samples at 105°C in a convection oven for 24 h. Samples were cooled to room temperature in a dessicator with indicating Drierite for 30 min before weighing. Unless otherwise noted, all moisture contents are reported as percent dry basis (d.b.).

Data Analysis

The following parameters were calculated to analyze the relationship between moisture content and microbial activity: peak CER, or the maximum CER measurement for each bioreactor; time to peak CER, or the time corresponding to the peak; and cumulative CER at 12 days, which was determined by numerically integrating CER profiles using Kaleidagraph (Synergy Software, Reading, Pa.).

A sigmoidal relationship between microbial activity and moisture has been observed in composting studies (Haug, 1993). Therefore, the following sigmoidal relationship was used to represent peak CER and cumulative CER:

$$y = \frac{a \cdot \exp\left[b(x - x_o)\right]}{1 - \frac{a}{c}\left(1 - \exp\left[b(x - x_o)\right]\right)} \quad (2)$$

where y is peak CER or cumulative CER, x is the natural logarithm of moisture content, x_o represents the natural logarithm of the lowest moisture content examined in the study, and a, b, and c are constant parameters. Parameter a was found by taking the average of the peak CER or cumulative CER values for all measured moisture contents below 30%, representing a baseline value for y. Parameters b and c represent the rate of increase in activity with increasing moisture and the maximum activity, respectively. Both parameters were found by fitting data to equation 2 using non-linear regression in Kaleidagraph.

Lag time for microbial activity was expected to decrease as initial moisture content increased. Therefore, a two-parameter exponential decay equation with respect to moisture was used to represent the data:

$$P_t = d \cdot \exp\left[-e(x - x_0)\right] \quad (3)$$

where d and e are constant parameters representing the initial amplitude of the curve and the rate of exponential decay, respectively. Both parameters were found by fitting data to equation 3 using non-linear regression in Kaleidagraph. Data were not included for bioreactors that showed no microbial activity.

Results

In this study, microbial activity was determined using respiration measurements made on straw between 15% and 200% moisture. An example data set collected at 35°C is shown in figures 1 and 2. There was little to no activity for the lowest two moisture contents of 15% and 23% (data not shown). For moisture contents between 26% and 43%, there

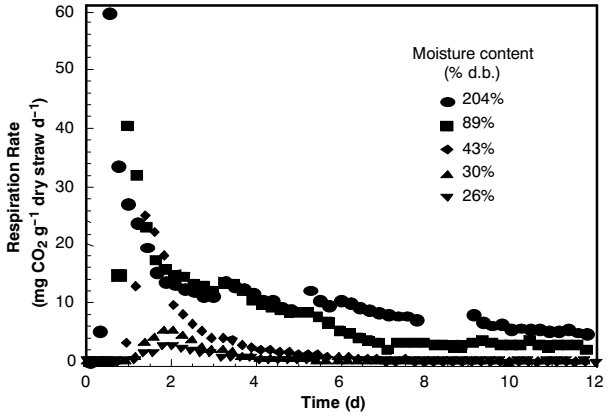

Figure 1. Respiration rate profiles for rice straw incubated at 35˚C at various moisture contents.

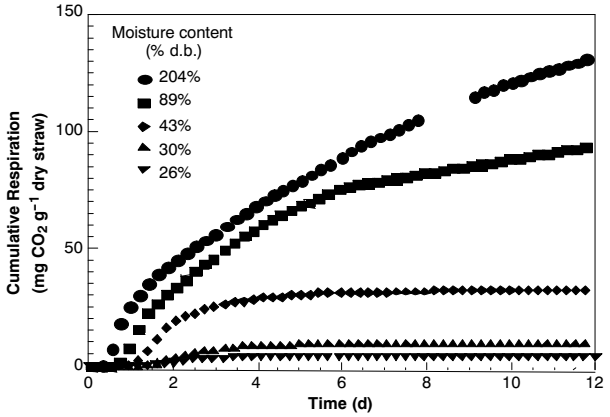

Figure 2. Cumulative respiration profiles for rice straw incubated at 35˚C at various moisture contents.

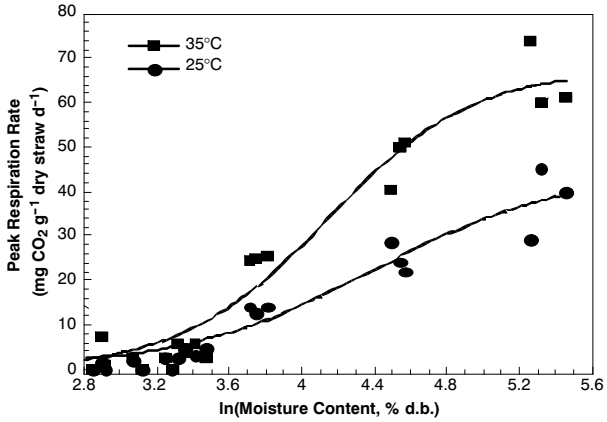

Figure 3. Increase in peak CER with moisture content for rice straw incubated at 25˚C and 35˚C. The lines represent curve fits with the sigmoidal relationship (eq. 2).

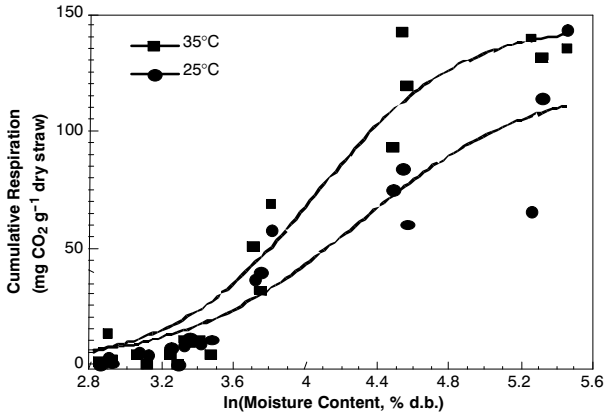

Figure 4. Increase in cumulative respiration with moisture content for rice straw incubated at 25˚C and 35˚C. The lines represent curve fits with the sigmoidal relationship (eq. 2).

was a brief lag in respiration rate followed by a small to medium peak that declined to a steady state close to zero (fig. 1). The highest moisture contents examined (89% and 204%) showed the most activity with the shortest lags, highest peaks, and had activity that declined to a steady state that was above zero. The trends in cumulative respiration were similar with increasing activity at moisture contents above 23% (fig. 2). The cumulative respiration leveled off after 5 days for moisture contents between 26% and 43%, while the curves continued to increase for the two highest moisture contents.

The two activity indicators, peak CER and cumulative CER, were plotted against moisture content to examine how activity changed with increasing moisture (figs. 3 and 4, respectively). Moisture content was transformed using the natural logarithm to facilitate data symmetry. Both indicators followed a sigmoidal relationship with activity increasing between approximately 25% and 80% moisture (corresponding to 3.2 to 4.4 on the natural logarithm scale). Peak CER from the 25˚C and 35˚C experiments (fig. 3) fit the sigmoidal equation (eq. 2) with R^2 values of 0.97 and 0.98 for 25˚C and 35˚C, respectively (table 1). Cumulative respiration at 12 days (fig. 4) also followed the sigmoidal relationship with R^2 values of 0.93 and 0.97 for 25˚C and 35˚C, respectively (table 1). The parameter representing the increase in peak CER or cumulative respiration with moisture (*b*) was higher at 35˚C compared to 25˚C. In addition the parameter indicating maximum peak activity or cumulative respiration (*c*) was greater at 35˚C than at 25˚C.

The amount of time required to achieve peak activity was also plotted with respect to the natural logarithm of moisture

Table 1. Parameter estimates and R^2 values for sigmoidal fits (eq. 2) of microbial activity indicators as a function of moisture content.

		Parameter *a*[a]		Parameter *b*[a]		Parameter *c*[a]		
Dependent Variable	Temperature	Value	Error	Value	Error	Value	Error	R^2
Peak CER	25°C	1.8	2	1.9	0.14	45	4.8	0.97
	35°C	1.8	2	2.5	0.14	68	4.0	0.98
Cumulative respiration	25°C	4.5	4	2.0	0.21	122	16	0.93
at 12 days	35°C	4.5	4	2.5	0.17	145	9.3	0.97

[a] Parameters *a* and *c* had units of mg CO_2 g-1 dry straw day-1 for regression of peak CER and mg CO_2 g-1 dry straw for regression of cumulative respiration. Parameter *b* was unitless.

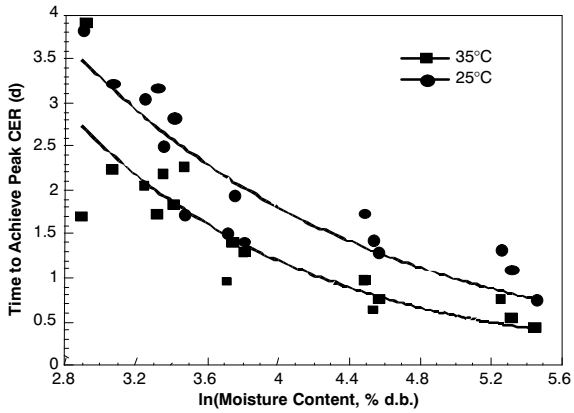

Figure 5. Time to achieve peak CER with respect to moisture content for rice straw incubated at 25˚C and 35˚C. The lines represent curve fits with the two-parameter exponential decay relationship (eq. 3).

Table 2. Parameter estimates and R^2 values for two-parameter exponential decay fits (eq. 3) for time to achieve peak CER as a function of moisture content.

	Parameter *d* (days)		Parameter *e* (unitless)		
Temperature	Value	Error	Value	Error	R^2
25°C	3.94	0.32	0.60	0.09	0.90
35°C	3.16	0.37	0.74	0.15	0.85

content (fig. 5). Straw at moisture contents less than 30% and at 25˚C took approximately 78 h to reach peak activity, while straw at the same moisture content at 35˚C took only 48 h. Straw above 30% moisture content took on average 36 h and 20 h to reach peak activity at 25˚C and 35˚C, respectively. The trend of time to peak versus the natural log of measured moisture content followed an exponential decay relationship (fig. 5) with R^2 values of 0.90 and 0.85 for 25˚C and 35˚C, respectively (table 2). The parameter representing the initial amplitude of the curve (*d*) was greater for storage at 25˚C compared to 35˚C. However, the parameter indicating the rate of decay in time to achieve peak CER (*e*) was equivalent for both temperatures.

DISCUSSION

Moisture levels that pose a risk for biomass deterioration and self-heating were identified based on respiration profiles and rate of initiation of microbial activity. The profiles for CER followed the expected trend for microbial growth in batch culture (Madigan et al., 2000; Shuler and Kargi, 1992). The sharpness of the peak in CER increased with increasing moisture. Sharp peaks have been observed in studies of self-heating due to microbial activity on straw and may be related to limited available substrate for the microorganisms (Carlyle and Norman, 1941). A higher peak in activity would translate to higher generation of waste metabolic heat and greater potential for biomass self-heating. The general increase in microbial activity with increasing moisture content has also been observed in hay (Festenstein et al., 1965; Rothbaum, 1963) and rice straw (Sain and Broadbent, 1975). The cumulative curves for the highest moisture contents did not level off to a constant value, indicating that microorganisms are able to degrade more substrate when more moisture is available, possibly because of a more active bacteria population or higher enzyme activities with higher moisture levels. The trends in cumulative activity indicate a greater risk of deterioration in biomass quality at higher moisture contents.

The sigmoidal trend of peak CER and cumulative CER with respect to moisture content was similar to observations made from composting studies (Haug, 1993). There is a threshold moisture content below which microbial activity will not occur and biomass will not deteriorate or self-heat. Above the threshold moisture content, some water is available for microorganisms to function, and microbial activity will increase with increasing moisture. There is an upper limit of moisture content beyond which microbial activity cannot increase due to other limiting factors, for example substrate or oxygen limitations. Maximum peak respiration and maximum cumulative respiration were greater at 35˚C compared to 25˚C, which is also consistent with composting behavior (Haug, 1993). The results demonstrate a greater risk for self-heating and biomass deterioration with increasing moisture and temperature.

The time to peak data followed an exponential decay; therefore, when more water is available, microorganisms become active more quickly. In biomass storage systems, this suggests that biomass wetting events must be mitigated quickly if deterioration and thermal runaway are to be prevented. Faster response times with increasing moisture suggest the importance of bacteria in this system. Further work is underway to determine the microbial communities responsible for this activity. The warmer temperature did stimulate microbial activity faster than the cooler temperature under the same substrate and moisture conditions. This indicates a greater risk of more rapid decomposition as the biomass self-heats.

CONCLUSIONS

Quality deterioration, dry matter loss, and increased risk of spontaneous combustion as a result of improper moisture control are critical concerns for storage of biomass. Based on this study, biomass deterioration is most likely at moisture contents greater than 70% dry basis where continuous microbial activity is not limited by moisture. Furthermore, based on higher peak and cumulative activities observed at 35˚C compared to 25˚C, the risk of deterioration is also greater at warmer temperatures. Stored biomass that experiences a rapid increase in activity, high peak activity, and that has limited cooling potential would have the greatest risk for self-heating and thermal runaway (Summers, 2005). Based on results from this study, these conditions would occur at moisture contents in the range of 50% to 80% dry basis, where moisture is great enough to stimulate rapid and sustained microbial activity but the evaporation potential and associated cooling is low. While the effect of moisture on microbial activity is greater at 35˚C than at 25˚C, the insulating effects of straw would still allow bales to heat quickly at lower temperatures. Energy balances on straw bales that allow temperature to be predicted during storage are needed to elucidate the role of moisture on self-heating and allow management decisions to be made to prevent rapid heating.

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