Available online at www.sciencedirect.com

ScienceDirect

Bioresource Technology 99 (2008) 6365–6371

BIORESOURCE TECHNOLOGY

Mold appearance and modeling on selected corn stover components during moisture sorption

C. Igathinathane [a], A.R. Womac [b,*], L.O. Pordesimo [a], S. Sokhansanj [c]

[a] *Agricultural and Biological Engineering Department, 100 Moore Road, Mississippi State University, Mississippi State, MS 39762, USA*
[b] *Department of Biosystems Engineering and Soil Science, The University of Tennessee, 2506 E.J. Chapman Drive, Knoxville, TN 37996, USA*
[c] *Oak Ridge National Laboratory, Environmental Sciences Division, Oak Ridge, TN 37831, USA*

Received 23 April 2007; received in revised form 29 November 2007; accepted 29 November 2007
Available online 28 January 2008

Abstract

Occurrence of mold was visually monitored for 26 days on samples of major anatomical components of corn stover maintained at several storage temperatures (T) and water activities (a_w). Glass desiccators with saturated salt solutions placed in temperature controlled chambers provided simulated storage conditions with temperatures ranging from 10 °C to 40 °C and water activities ranging from 0.11 to 0.98. Mold affected leaf, stalk skin, and stalk pith equally at water activity greater than 0.9. As expected, a combination of increased water activity greater than 0.9 and temperatures greater than 30 °C was conducive to mold growth. Based on material moisture content during the initial mold growth, it was postulated that among the corn stover components the stalk pith was the least resistant to mold growth followed by stalk skin and leaf for the studied range of temperature and water activity. Mold growth models fitted well with the observation. A linear mold-free days predictions using a three-parameter regression model (T, a_w, and $T \times a_w$) was superior ($R^2 = 0.99$) to other models considered. The exponential spoilage model using two parameter T and a_w also gave comparable performance ($R^2 = 0.95$). Among the independent factors, $T \times a_w$ product was the most significant ($p = 0.0069$) followed by T ($p = 0.0114$), and a_w ($p = 0.3140$) in explaining the experimental data. The developed models can be applied to predict the safe storage period of corn stover components exposed to various temperature and moisture environmental conditions.

Keywords: Biomass; Corn stover; Isotherms; Mold growth model; Water activity

1. Introduction

Biomass or cellulosic plant materials have potential to supply future energy needs (USDA, 2007). Corn stover along with other collectable residues and herbaceous energy crops-like switchgrass were identified as alternative feedstock that will supplement the feedstock demands (Sokhansanj et al., 2002; McLaughlin and Kszos, 2005). Based on one estimate (Kim and Dale, 2004), globally about 203.6Tg of dry corn stover is available with a potential of producing about 58.6 GL of bioethanol, replacing about 3.8% of world annual gasoline consumption; and the additional energy generated from the lignin-rich fermentation residues equals about 0.7% of total global electricity generation. Safe storage and preprocessing of biomass are important operations in supplying biomass to conversion facilities.

Although microorganisms are useful in biological conversion of plant wastes into value added products in controlled conditions, mold growth on the material during storage before actual bioconversion procedures is undesirable (Essien et al., 2005). In addition, mold growth on biomass presents health hazards to operators. Mold mycelium and mold spores are products of fungal growths that thrive on wet and warm organic matters (CRDBER, 2005). Biomass is expected to be stored in ambient atmospheric conditions with exposure to mold from the field or mold coming in contact during storage. The storage can be in an open

---

[*] Corresponding author. Tel.: +1 865 974 7104; fax: +1 865 974 4514.
E-mail address: awomac@utk.edu (A.R. Womac).

0960-8524/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2007.11.075

field or in an enclosure. Determining environmental temperature and moisture conditions that cause mold growth on stored corn stover helps in developing efficient storage systems and scheduling supply and storage strategies.

Corn stalk and leaf contain a large portion of the above ground stover mass (grain with cob intact is excluded) and the rest being husk component. Corn stalk and leaf represent 72.6% and 20.7%, respectively, the wet mass of stover (Igathinathane et al., 2006). Similarly on a dry matter basis of stover, stalk and leaf represent 60% and 25%, respectively (Pordesimo et al., 2004). Analyzing corn stalk and leaf thus provide a representative sample of the bulk of the mass from the corn plant. Exposure of stalk skin and stalk pith may occur due to crushing, twisting, and pulling actions of combine harvesters that expose stalk pith otherwise tightly enclosed by stalk skin. Partitioning stalk skin and pith components may be helpful in microbial growth observations. Mold growth studies on corn stover components under controlled conditions are not available in the current literature. The objectives of this paper are to describe experimental observations on the mold growth on leaf, stalk skin, and stalk pith; and to develop regression models to predict the mold-free days of mold infestation on samples.

2. Methods

Igathinathane et al. (2005) reported on the development of mold while determining sorption isotherms of corn stover leaf, stalk skin, and stalk pith components. The present work is a part of the above study to determine equilibrium moisture contents of corn stover components during sorption using static method with saturated salt solutions. This research is meant to establish the preliminary environmental conditions on controlled settings which support the mold growth on corn stover components assessed visually, and not directly meant to address issues of storage of dry biomass or wet biomass as silage. Samples were exposed to air-tight environment and these conditions were presumably different from the complete anerobic conditions of wet silage and partial anerobic conditions of loose pile or bales of biomass. Therefore, the results may not directly relate to these biomass storage scenarios.

Prepared samples of field-grown Dekalb 743 corn variety with components having moisture content of 0.06–0.12 decimal dry basis (d.b.) were subjected to a series of temperatures and water activities (equilibrium relative humidity) for about a month. Six temperatures (T) ranging from 10 °C to 40 °C at intervals of 5 °C were considered. Ten saturated salt solutions in glass desiccators maintained water activities (a_w) from 0.11 to 0.98 (Table 1). A hot air convection oven maintained temperatures $\geqslant 30$ °C for those treatments. A refrigerated incubator maintained temperatures <30 °C for remaining treatments. Sample preparation details, and sample physical dimensional information were already reported (Igathinathane et al., 2005). Samples were cut from random corn plants to randomize mold spore source.

Table 1
Water activity of saturated salt solutions at 10 °C and 40 °C (Greenspan, 1977)

Saturated salt solution	Water activity (decimal) at 10 °C and 40 °C
Lithium chloride	0.113–0.112
Potassium acetate[a]	0.234–0.189
Magnesium chloride	0.335–0.316
Potassium carbonate[a]	0.431–0.431
Magnesium nitrate	0.574–0.484
Potassium iodide	0.721–0.661
Sodium chloride	0.757–0.747
Potassium chloride	0.868–0.823
Potassium nitrate[b]	0.960–0.890
Potassium sulfate	0.982–0.964

[a] 35 °C and 40 °C a_w values are extrapolated from the available 10–30 °C a_w values.

[b] Not used at 10 °C.

Mini glass beakers (10 ml capacity, 22 mm inner diameter and 33 mm height) stored inside glass desiccators held samples of corn stover components (Fig. 1). The beakers were washed and chemically (ethyl alcohol) sterilized before use. Three replications each for leaf, skin, and pith for a total of nine mini glass beakers were arranged on a stainless steel base plate inside each desiccator. Each beaker held samples (6–10 pieces) with total weight of approximately 0.2 g of leaf, 0.8 g of stalk skin, and 0.15 g of stalk pith. During experiments, microbial growth inhibitors like phenyl mercury acetate or thymol, which are common in sorption studies (Rahman, 1995) were not included in the desiccators to allow for the growth of mold from field infestation. Samples were regularly observed for visible spores and subsequent mycelium formation (white to greenish cloudy appearance) on the surface, and the weights of the samples with beakers were recorded daily. From the initial moisture content and initial and final sample weights, the sample moisture contents during experiments were calculated. During the experiments CO_2 production and subsequent dry matter loss was not measured or estimated. The observed weight differences were assumed to be only related to moisture transfer, and this assumption accounts for the possible dry matter loss. The weight measurements of each sample was taken by briefly opening the desiccator lid, taking out the mini beaker with sample, closing the lid back, taking weight measurement, and replacing the mini beaker by briefly opening and closing the lid again. The entire process of sample weight measurement took about 15 s. Even in this period, the samples were briefly exposed to the ambient conditions of room only during the opening and closing of the desiccator lid. This brief exposure of samples to ambient conditions has not altered the equilibrium moisture values and mold growth characteristics judging from the absence of abrupt variation on moisture sorption and non-appearance of mold on samples exposed to reduced temperatures and water activities.

The day of first appearance of mold, in the form of visible network of mycelium on the samples, with days counted from the start of experiment, was recorded.

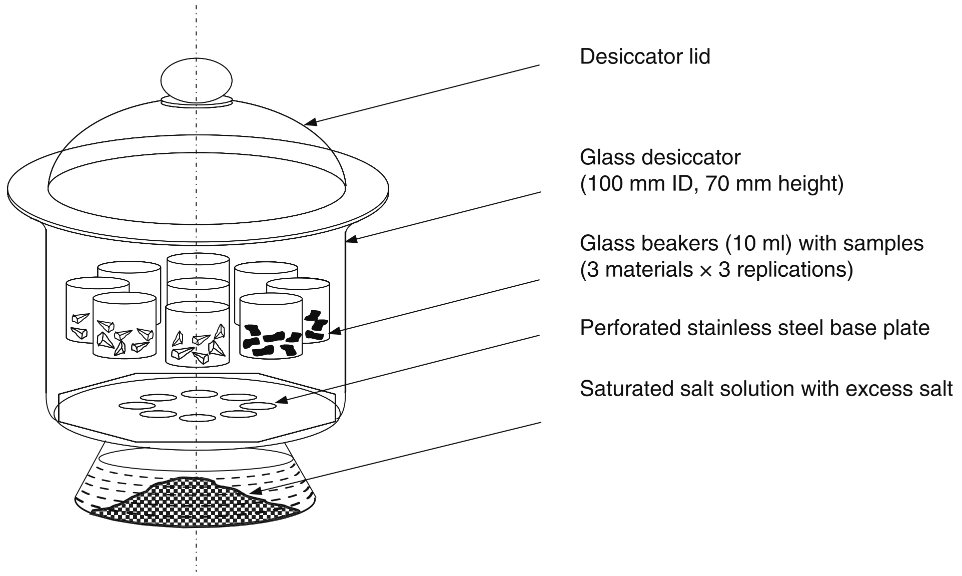

Fig. 1. Mold growth on corn stover components observation during moisture sorption using glass desiccator controlled environment.

Elapsed days before mold appearance were herein termed as mold free days (MFD) for modeling purposes. Numerically, MFD is one less than the days counted to the first mold appearance. Although mold formation as small black dots was observed a few days prior to the cloudy appearance of mold, those days were not considered in the analysis of this study. Samples exhibiting mold were not disturbed during weight measurements. The naturally occurring species of mold were not identified and the mold population was not counted. The mini-beakers with samples exhibiting mold were not isolated in order not to interfere with the sorption isotherm experiments.

Mold propagated fast with increased temperature and equilibrium water activity. Working with pelletized corn grain based animal feed and alfalfa cubes, respectively, Herrman and Loughin (2003) and Sokhansanj et al. (2003) separately proposed an exponential spoilage model for MFD in terms of temperature and water activity. In the present study, models with a few linear combinations of temperature and water activity were developed along with the reported exponential model and their prediction performances were compared by inspection of coefficient of determinations. The NLIN procedure of SAS (2002) fitted the multiple non-linear regression models, and *t*-test probabilities determined the model significant factors.

Independent parameters, namely, temperature, water activity, and their interaction ($T \times a_w$) influencing the mold growth were combined in mathematical models to estimate MFD. The models considered were Model 1: Three-parameter model involving interaction ($T, a_w, T \times a_w$); Model 2: Two parameter model (T, a_w); Model 3: Single parameter model of $T \times a_w$ product; and Model 4: Two parameter (T, a_w) reported exponential model (Herrman and Loughin, 2003; Sokhansanj et al., 2003; Khoshteghaza et al., 1999a,b).

3. Results and discussion

3.1. Mold growth observation

Contour plots of equal MFD's at several combinations of temperature and water activity data are shown in Fig. 2. MFD observations were enclosed by two clear limiting "Region-A" of no mold growth and "Region-B" of earliest mold growth. Region-A represented no visually observable mold growth during the experimental period with MFD >26; and its environmental conditions were $T \leqslant 30$ °C with $a_w \leqslant 0.96$. It should be noted that mold growth would occur even in the safe Region-A if the study period were extended much beyond 26 days, and further research establishes the exact MFD with extended storage. Region-B of earliest mold growth having MFD $\leqslant 8$ was a triangular region with environmental conditions of $T \geqslant 30$ °C with $a_w \geqslant 0.97$. A narrow band, between these regions, covered the entire observation. At reduced temperatures ($T < 30$ °C), the MFD contours were densely packed near $a_w > 0.96$. For the increased temperatures ($T \geqslant 30$ °C), MFD contours were more uniformly distributed for water activities $\leqslant 0.96$. MFD contours were densely distributed in a narrow range of $0.965 \leqslant a_w \leqslant 0.975$ with $T \geqslant 30$ °C, and uniformly outside this range.

Any combination of $T \leqslant 30$ °C with $a_w \leqslant 0.96$ can be recommended for safe storage of corn stover components for at least 26 days (MFD > 26, Region-A). Herrman and Loughin (2003) also found that cooler temperature would take longer to develop mold. Specifically, at $a_w = 0.70$ the corn feed pellets held at 25 °C, 30 °C, and 35 °C had 53, 36, and 32 MFD, respectively. Based on visual appearance of mold growth, alfalfa cubes shipped from Canada to Taiwan had a wide range of 7–42 MFD (Sokhansanj et al., 2003) depending on combination of T and a_w.

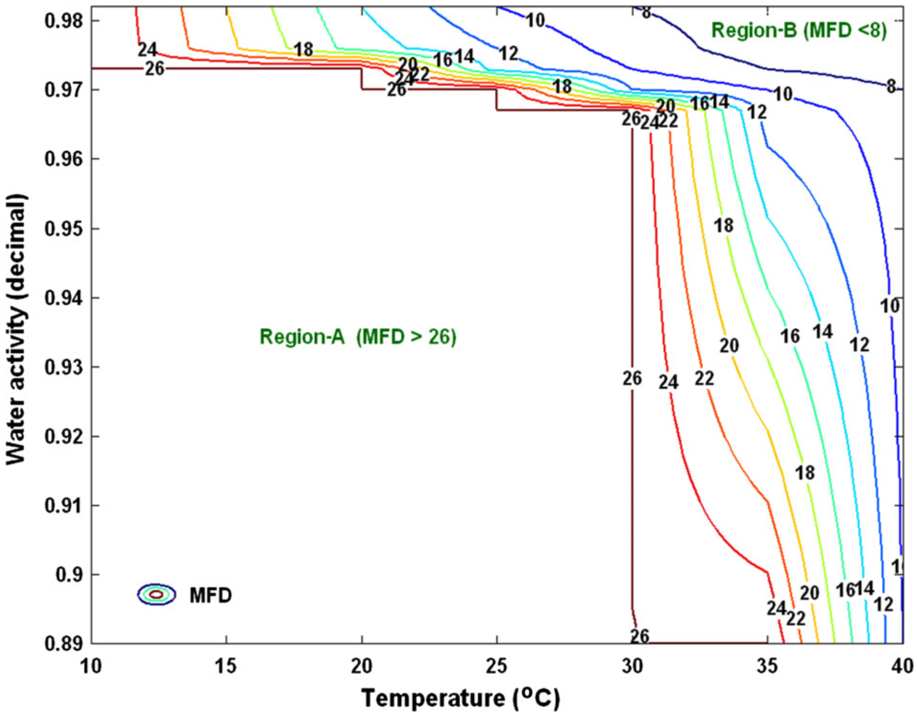

Fig. 2. Observed mold growth expressed as mold free days (MFD) on corn stover samples. MFD observations were enclosed by two clear limiting "Region-A" of no mold growth and "Region-B" of earliest mold growth.

Mycelium growth was noticed as early as seven days on samples subjected to greater temperature and water activity. Once appeared on samples, mold growth was sustained throughout the experimental period. Inside a given desiccator, no difference on mold growth progression was observed among the corn stover components, as mold growth was found on all the samples equally. Samples being kept in the same desiccator may have experienced increased levels of available spores after mold growth accelerated on other samples within the same desiccator. On the other hand, since mold spores are assumed to be omnipresent on all samples, independent mold growth can begin and be attributed primarily to favorable temperature and moisture conditions. Further investigation by separating the corn stover components stored separately in individual desiccators would be required to address whether accelerated mold growth on one component could affect growth on other components. However, this expanded approach would only be required if commercial practice fractionated corn stover components for storage, as these components exist intact in their natural state even after preprocessing size reduction.

The lower limits of water activity at corresponding temperatures when mold growth was first observed on samples are presented in Table 2. Water activities greater than the tabulated values at corresponding temperatures also sustained mold growth on the samples. The entire range of $a_w < 0.90$ was mold free at all temperatures. Reduced temperatures and reduced water activities suppressed mold growth; however, mold growth was suppressed at increased temperatures when the water activity was reduced. Similarly, at reduced temperatures the increased range of water activity corresponded with mold growth suppression. Moreover, any other condition of temperature and water activity lower than the shown limits (Table 2) would extend the MFD beyond the tabulated values.

Table 2
Storage conditions of corn stover one day after MFD

T (°C)	a_w[a] (decimal)	$T \times a_w$ (°C)	MFD[b] (day)
40	0.890	35.60	10
35	0.967	33.85	11
30	0.970	29.10	12
25	0.973	24.33	13
20	0.976	19.52	15
10	0.982	9.82	26

[a] Minimum a_w at which mold growth was observed.

[b] MFD applies to all corn stover components, namely, leaf (0.199–0.468 d.b.), stalk skin (0.182–0.432 d.b.), and stalk pith (0.178–0.426 d.b.).

Product of temperature and water activity ($T \times a_w$, interaction term) served as a comprehensive single parameter to monitor the mold growth. The product of $T \times a_w$ decreased from 35.60 °C to 9.82 °C as MFD increased from 10 to 26 (Table 2). For mold-free long duration storage of the corn stover components, the value of $T \times a_w$ product should be kept as low as possible within practical limits.

Moisture contents of corn stover component samples on the day before the visible mold growth were determined and plotted against $T \times a_w$ product (Fig. 3). At the start of visible mold growth, the instantaneous moisture content of the leaf (0.199–0.468 d.b.) was the greatest followed by

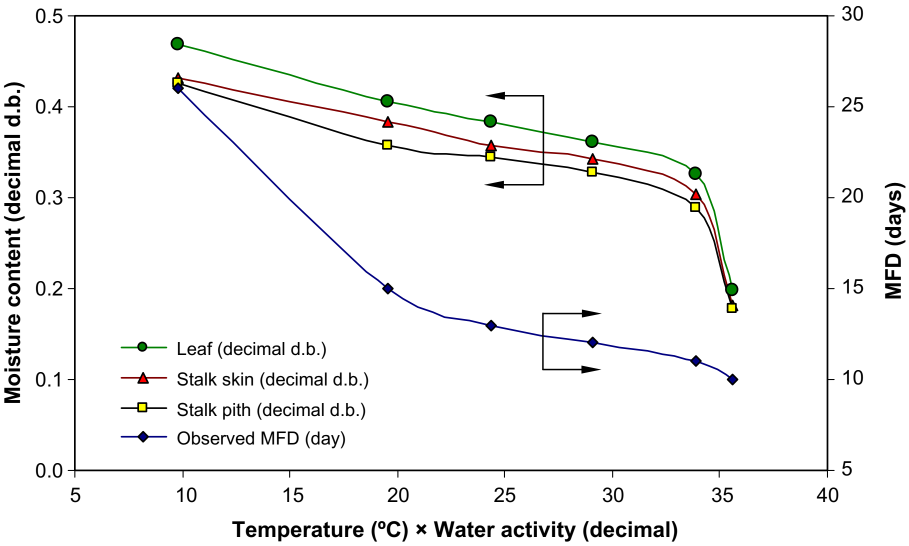

Fig. 3. Moisture content of corn stover components with corresponding MFD.

stalk skin (0.182–0.432 d.b.) and stalk pith (0.178–0.426 d.b.) for different T and a_w. The observed equilibrium moisture content ranges were 19.9–56.4%, 18.2–41.1%, and 19.5–71.5% d.b. for corn leaf, stalk skin, and stalk pith, respectively in the mold forming $a_w > 0.89$ (Igathinathane et al., 2005). It should be noted that the moistures reported in Fig. 3 are the instantaneous moisture on the day of initial mold growth at various T and a_w, and were different from the equilibrium moisture content values discussed above. The relative moisture equilibrium of the components provided stable moisture for mold growth. As $T \times a_w$ product increased, mold growth occurred even at low moisture levels.

Notwithstanding the possible elevated levels of spores after accelerated growth on other components in the desiccator, it can be postulated that the stalk pith recording the lowest moisture as the least mold resistant corn stover component followed by stalk skin and leaf (Fig. 3) based on the components instantaneous moisture. Considering increased moisture promotes mold growth, the instantaneous moisture content of the component, at which the initial visible mold growth was observed, can be thought of the favorable lower limit of moisture for molds and their growth will be sustained at moistures above this limit. For instance, the stalk pith instantaneous moisture content at initial visible mold growth was 0.426 d.b. at 10 °C T and $0.982 a_w$, and any moisture greater than this value would have also supported mold growth. However, at the same conditions of T and a_w the moistures of stalk skin and leaf were 0.432 and 0.468 d.b., respectively, which may indicate that at any lower instantaneous moisture mold would not have grown on them. Similar trend were observed with other T and a_w values (Fig. 3). Therefore, stalk pith having the wider range of moistures was concluded to be more susceptible for mold growth compared to stalk skin and leaf. Stalk pith chemical composition is not drastically different as its major sugar contents such as glucan and xylan of stalk pith were between stalk skin and leaf, although lignin content of stalk pith is the least among the components (Ye et al., 2006). However, physically the structure of stalk pith is spongier than stalk skin and leaf. Further research efforts are required to confirm and fully address the reasons why mold grows over a wider moisture contents on stalk pith.

3.2. Modeling of MFD

Model predictions are represented as a plot of MFD versus $T \times a_w$ to determine their performance (Fig. 4). The fitted models and their performances are

Model 1	$\mathrm{MFD} = -1925.30 + 47.51T + 1991.04a_w - 48.80Ta_w$	$(R^2 = 0.99)$
Model 2	$\mathrm{MFD} = 83.30 - 0.62T - 54.42a_w$	$(R^2 = 0.84)$
Model 3	$\mathrm{MFD} = 28.89 - 0.57Ta_w$	$(R^2 = 0.87)$
Model 4	$\mathrm{MFD} = \exp(7.10 - 0.04T - 3.52a_w)$	$(R^2 = 0.95)$

where MFD is mold free days in days, T is temperature in Celsius, and a_w is water activity as a decimal.

Since all samples inside desiccators were found moldy around the same period, the developed relationships apply equally to all corn stover components studied in their respective moisture range (Table 2). The three-parameter model (Model 1; $R^2 = 0.99$) gave the best prediction compared to the exponential model (Model 4; $R^2 = 0.95$)

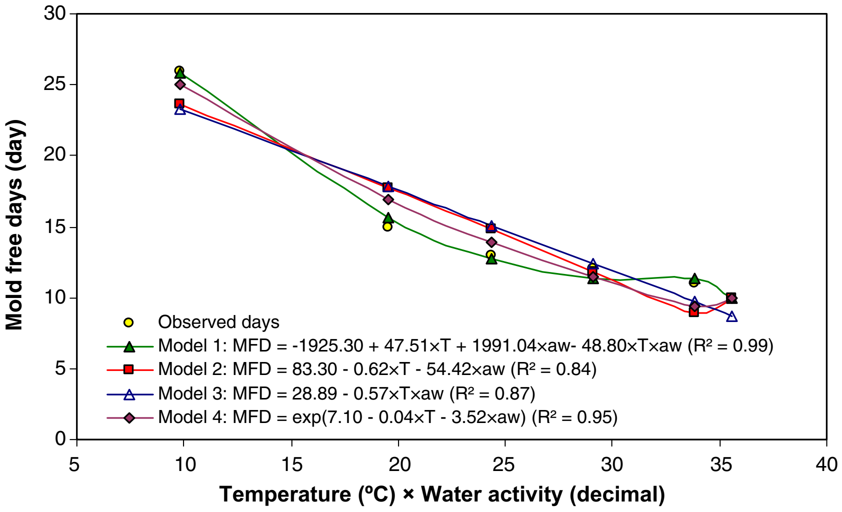

Fig. 4. Comparison of observed and predicted MFD of the corn stover components.

proposed by Herrman and Loughin (2003) and Sokhansanj et al. (2003). The single parameter $T \times a_w$ product model (Model 3; $R^2 = 0.87$) produced slightly better results than the two parameter model (Model 2; $R^2 = 0.84$). From Model 1 featuring all parameters, the *t*-statistics revealed that the combined $T \times a_w$ product variable ($t = -5.11$, $p = 0.0069$) was found to be more significant followed by T ($t = -4.43$, $p = 0.0114$), and the least significant variable was a_w ($t = 1.15$, $p = 0.3140$). These models, demonstrating good fit of MFD, can be utilized to predict the safe storage period of corn stover components from temperature and relative humidity of the storage environments within the storage period studied. It should also be noted that the spore formation, mold growth rate, length of storage period, and extent of spread would also be influenced by other environmental conditions, such as, type and species, spore availability, material types and composition, lighting condition, air movement, and transient and cyclic temperature and moisture variation (CRDBER, 2005). Further research is needed to address transient and cyclic temperature, longer duration of storage, and moisture variations common to most commercial biomass storage facilities.

4. Conclusions

All corn stover components were affected by mold at water activity greater than 0.90. For a given water activity, increased temperatures in the studied range (10–40 °C) were found to be conducive to mold growth. During initial mold growth the moisture content of leaf was the greatest followed by stalk skin and stalk pith. Based on range of moisture favorable to mold growth, it can be postulated that stalk pith was the least resistant component to mold growth followed by stalk skin, and leaf. When conditions were conducive and corn stover components stored together, initial mold growth occurred on all components at the same day. Mold growth models revealed that product of temperature and water activity was the most significant ($p = 0.0069$) variable followed by individual temperature ($p = 0.0114$), and water activity ($p = 0.3140$) variables. The mold growth models expressing the number of mold free days as a function of temperature and water activity gave good fit and they can be utilized to assess storage of dry corn stover under different storage conditions.

References

CRDBER, 2005. Conceptual Reference Database for Building Envelope Research – Essay: Molds. <http://alcor.concordia.ca/~raojw/crd/essay/essay000073.html> (accessed 28.01.2007).

Essien, J.P., Akpan, E.J., Essien, E.P., 2005. Studies on mould growth and biomass production using waste banana peel. Bioresource Technology 96, 1451–1456.

Greenspan, L., 1977. Humidity fixed points of binary saturated aqueous solutions. Journal of Research of the National Bureau of Standards, Section A. Physics and Chemistry 81, 89–102.

Herrman, T.J., Loughin, T., 2003. Processing and shelf-life performance of feed manufactured from high-moisture corn. Transactions of the American Society of Agricultural Engineers 46, 697–703.

Igathinathane, C., Womac, A.R., Sokhansanj, S., Pordesimo, L.O., 2005. Sorption equilibrium moisture characteristics of selected corn stover components. Transactions of the American Society of Agricultural Engineers 48, 1449–1460.

Igathinathane, C., Womac, A.R., Sokhansanj, S., Pordesimo, L.O., 2006. Mass and moisture distribution in aboveground components of standing corn plants. Transactions of the American Society of Agricultural Engineers 49, 97–106.

Kim, S., Dale, B.E., 2004. Global potential bioethanol production from wasted crops and crop residues. Biomass and Bioenergy 26, 361–375.

Khoshteghaza, M.H., Gossen, B.D., Sokhansanj, S., 1999a. Predicting molding of alfalfa cubes in transit. Canadian Journal of Plant Pathology (abstract) 21, 195.

Khoshteghaza, M.H., Sokhansanj, S., Gossen, B.D., 1999b. Quality of alfalfa cubes during shipping and storage. Applied Engineering in Agriculture 15, 671–676.

McLaughlin, S.B., Kszos, L.A., 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. Biomass and Bioenergy 28, 515–535.

Pordesimo, L.O., Edens, W.C., Sokhansanj, S., 2004. Distribution of aboveground biomass in corn stover. Biomass and Bioenergy 26, 337–343.

Rahman, S., 1995. Food Properties Handbook. CRC Press, Boca Raton, Florida.

SAS, 2002. SAS version 9 online help and documentation. Cary, N.C.: SAS Institute, Inc.

Sokhansanj, S., Turhollow, A., Cushman, J., Cundi, J., 2002. Engineering aspects of collecting corn stover for bioenergy. Biomass and Bioenergy 23, 347–355.

Sokhansanj, S., Khoshtaghaza, H., Schoenau, G.J., Arinze, E.A., Tabil, L.G., 2003. Heat and moisture transfer and quality changes in containerized alfalfa cubes during transport. Transactions of the American Society of Agricultural Engineers 46, 423–432.

USDA, 2007. USDA News release. Release No. 0012.07. <http://www.usda.gov/wps/portal/!ut/p/_s.7_0_A/7_0_1OB?contentidonly=true&contentid=2007/01/0012.xml> (accessed 28.01.2007).

Ye, X.P., Liu, S., Kline, L., Hayes, D.G., Womac, A.R., Sokhansanj, S., Narayan, S., 2006. Fast biomass compositional analysis using Fourier Transform Near-infrared Technique. ASABE Paper No. 066155. St. Joseph, Michigan: ASABE.