

# EFFECTS OF COMPRESSION ON PARENCHYMA CELL VIABILITY, INITIAL HEATING, AND MICROFLORA OF ASPEN FUEL CHIPS<sup>1</sup>

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## ABSTRACT

In order to study fundamental factors influencing storage of compression-dewatered wood chip bales, compressed bigtooth aspen chips were compared to noncompressed chips to note differences in parenchyma cell viability, initial heating, and early microflora types. Immediately after pressing (28 MPa for 5 min), surface area of chips stained as a result of parenchyma cell dehydrogenase activity on triphenyltetrazolium chloride was reduced from 83% (noncompressed) to 23% (compressed). Half-life of remaining viable parenchyma cells was reduced by 14 days at ambient temperatures. Temperatures in insulated chip bales were 30% higher for compressed chips after 24 h than in noncompressed chips. After 10 days of ambient storage, bacteria were abundant in the compressed chips, while *Phycomycetes* and *Fungi imperfecti* were more frequently isolated from the noncompressed chips. Such differences in compressed chips may influence the fuel potential of baled chips stored for extended periods by changing the pattern and parameters of organism activity.

**Keywords:** Wood chips, bigtooth aspen, compression, parenchyma viability, heating, microflora.

## INTRODUCTION

Heating and microbial activity in bulk piles of wood chips have been well documented (Feist et al. 1971, 1973; Springer and Hajny 1970), and management methods have been developed to reduce deterioration during storage (Fuller 1985). However, little is known concerning storage of baled green wood chips that have been compression-dewatered to increase their fuel value. The temperature, moisture, and microflora interactions in such baled chips must be better understood in order to minimize losses in fuel value and biomass (Steklenski et al. 1989). This study was conducted to assess the initial differences between compressed and noncompressed hardwood chips in parenchyma cell viability and longevity, heating of chips bales, and microflora patterns. Substantial differences in these factors might influence bale drying patterns, prevalence, and effects of wood-destroying fungi known to develop in bales (Steklenski et al. 1989), and overall storage ability of compression-dewatered chip bales intended for use as fuel.

## MATERIALS AND METHODS

Four 1,250-mm-long (4-feet) bolts with diameters ranging from 115–150 mm (4–6 inches) were cut from a single bigtooth aspen (*Populus grandidentata* Michx.) in mid-August. These bolts were chipped with a disc-chipper 5 days after cutting. Chips were thoroughly mixed and separated into two batches. One of the batches

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was pressed with a hydraulic ram press (Steklenski and Haygreen 1987) at 27.6 MPa (4,000 psi) for 5 minutes to produce compressed chips; the other remained noncompressed. Approximately 8 liters of both compressed chips and noncompressed chips were put into separate plastic bags and stored in a controlled environment (21 C, 50% RH). The plastic bags were covered loosely with aluminum foil to reduce moisture loss and to allow air exchange.

The viability of the parenchyma cells in the compressed and the noncompressed chips was tested by immersing the chips in a 1% TTC solution (1g of 2,3,5-Triphenyltetrazolium chloride [Sigma Chemicals] in 99g of distilled water) for 18 to 24 h (Feist et al. 1971). The appearance of red color on the chip surface indicated viable parenchyma cells in the chip (Koenigs 1966). Stained areas may also result from microorganism dehydrogenase activity on TTC. The stained areas on the chips caused by microorganisms were scattered small spots (purple-red in color), while those caused by viable parenchyma cells were larger confluent patches (pink to bright-red in color). The stain differences on the chip surface were also checked microscopically (400 $\times$ ); purple-red spots were stained bacterial cells (occasionally, fungal hyphae) readily distinguished from the pink- to red-colored vacuoles in the parenchyma protoplasm. A chip with no red color typical of parenchyma protoplasm was noted as one without viable parenchyma.

Samples of 30 randomly selected chips from each of the bags were chosen immediately after the compression of the chips (day 0), every day during the first week, every other day during the second week, and every three days after the second week of storage. The number of chips with any areas of visible red stain due to parenchyma dehydrogenase activity was recorded and divided by 30 to give the percentage of chips with viable parenchyma cells for each sample tested. Quadratic regressions were fitted, and the half-life (50% of chips with viable parenchyma cells) for compressed and noncompressed chips was estimated (Weisberg 1985).

The percentages of red-stained area of compressed and noncompressed chips at day 0 were determined by projecting photographic slides of these chips onto a digitizing pad and measuring red area using an image analysis system (Micro-Comp; Southern Micro Instrument, Inc.). A total of 5 subsets of 4 chips were measured for each set to determine the effect of compression on the viability of parenchyma cells.

Approximately 125 of each type of chip were placed into each of three 475-ml (16-oz) glass jars (covers loose for air exchange), and incubated in a controlled environment (21 C, 50% RH). The influence of compression on the developing microflora was estimated after 10 days of incubation. Because no microbial colonies were visible at this time to the naked eye, three chips were randomly selected from each jar and split into splinters. The surface of the splinter was surface disinfected in a flame and placed onto agar media and incubated at room temperature (24 C). Four types of media were used: malt extract agar (1.5% Difco malt extract and 2% Difco agar), malt extract agar with 20 ppm benomyl (generally selective for Basidiomycetes), malt extract agar with 20 ppm rose bengal, and cellulose agar (Eggins and Pugh 1962). Three splinters selected from the pooled chips from both the compressed and noncompressed sets were placed onto 2 plates of each type of medium. After one week of incubation, colonies of fungi

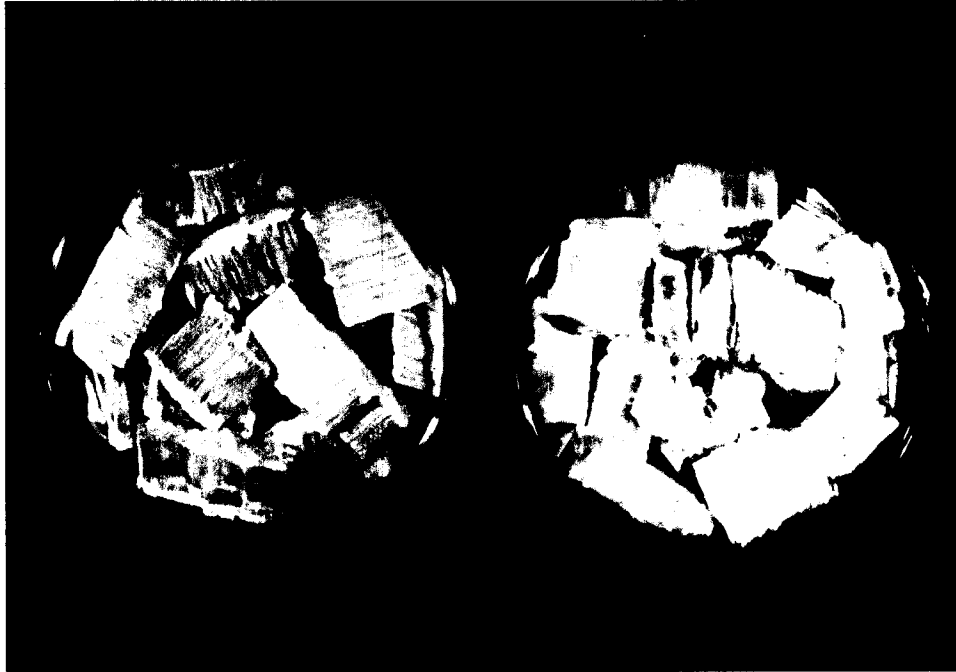


FIG. 1. TTC stained compressed chips (right) and noncompressed chips (left) chips.

and bacteria growing from the splinters on the media were counted, subcultured and further incubated for one month and then generally categorized as bacteria, Phycomycetes (with Mucoraceous type sporangia), *Fungi imperfecti* (mostly Aspergilli and dematiaceous Hyphomycetes, but some isolates could have been nonsporulating Ascomycetes), or Basidiomycetes (presence of clamp connections).

Some jars of compressed chips were rewetted with sterile water to the green moisture content (80% O.D. basis) and then incubated. The purpose of this parallel experiment was to investigate the effect of moisture content alone on the microflora of the compressed chips.

In order to determine the effects of compression on the initial heating of chips, compressed chips were made into bales (0.06 cubic meter [1.61 cubic feet], 275 Kg/cubic meter [17.1 pcf]) to simulate the situation in a wood chip pile. Three such bales were made and enclosed with 50-mm-thick polystyrene foam insulation ( $R = 8$ ). Noncompressed chips were packed into boxes constructed with the same polystyrene foam, which retained the same volume as the compressed chip bale. Thermocouples were inserted into the center of each chip pile simulator and the temperatures were recorded daily (Springer et al. 1969).

#### RESULTS

The TTC stained area of compressed chips was greatly reduced (Fig. 1). Measured stained surface areas for compressed and noncompressed chips at day 0 were 23.3% and 83.0%, respectively. The edges of both compressed and noncom-

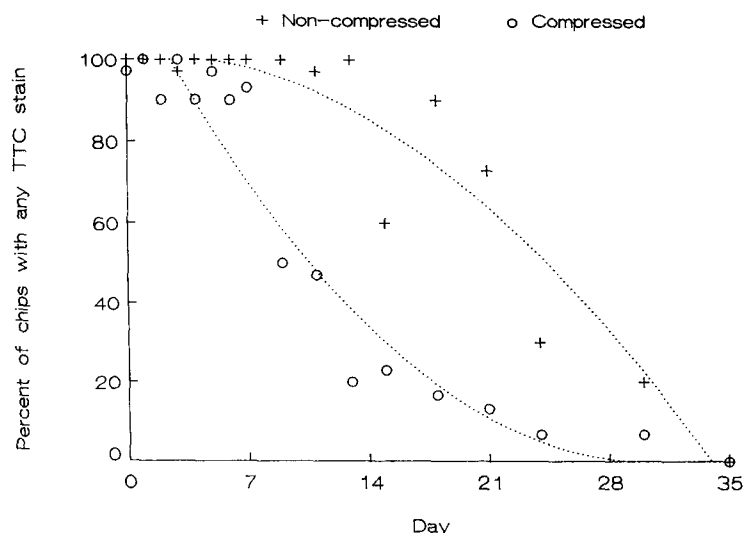


FIG. 2. Influence of compression on viability of parenchyma cells over time, quadratic regression lines fitted.

pressed chips were not stained. This may be due to the mechanical impact and resulting death of parenchyma cells along the edges during the chipping process.

The percentage of chips with any viable parenchyma staining for both compressed and noncompressed chips decreased over storage time. Both types of chips retained at least some measurable viability up to 35 days, which is similar to the 32 days for aspen chip viability reported by Feist et al. (1971). However, as shown in Fig. 2, the rate of decrease differed for the chip sets (significant at the 99.9% confidence level). The percentage of chips staining decreased more rapidly for the compressed chips. The estimations of the half-life were 24.5 days for the non-compressed chips and 10.6 days for the compressed chips.

The microorganisms isolated from the compressed and noncompressed chips after 10 days of incubation differed in categories and colony-forming frequency. For both types of chips and for the rewetted chips, about 25% of the incubated splinters developed colonies of microorganisms after one week. The compressed chips had a relatively high percentage of bacterial colonization (16.6% of the total incubated chips) along with some Basidiomycetes (8.3% of the total incubated chips). In contrast, 12.5% of the noncompressed chips provided Phycomycetes colonies and 8.3% of the chips colonies of probable *Fungi imperfecti*. The early stage microflora of the rewetted compressed chips were similar to that from the noncompressed chips (21% Phycomycetes and 4% *Fungi imperfecti* total isolation frequency).

The initial internal temperature after the first day of storage was the most distinguishable difference between the compressed and noncompressed chip bales. The compressed chip bales heated rapidly and reached a heating peak (42 C) after the first day of storage. The temperature of the noncompressed chip bales showed a steady increase with storage until, at day 4, both types of the bales had approximately the same temperature (32 C) (Fig. 3).

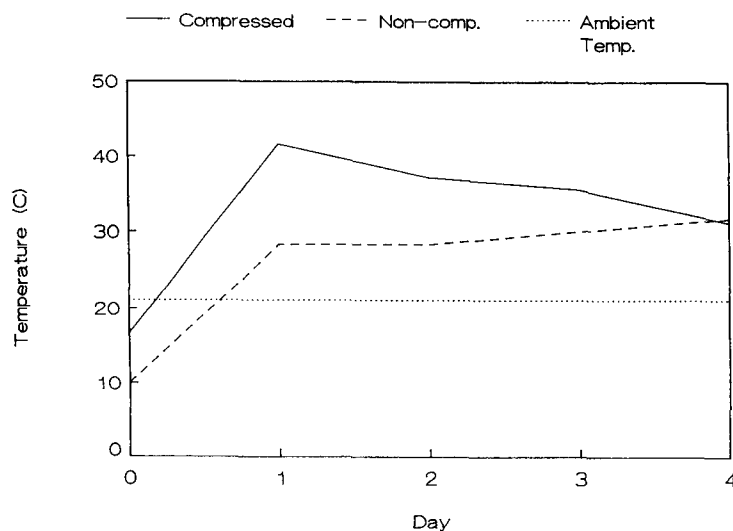


FIG. 3. Temperature profiles of compressed and noncompressed chips.

#### DISCUSSION

The direct effect of compression of aspen chips is to reduce the number of the viable parenchyma cells. Approximately 60% of the parenchyma cells were damaged during the compression process reducing the total viability of the parenchyma cells. In comparison to the noncompressed chips, the half-life of the parenchyma cells in the compressed chips was reduced by 14 days (from 24.5 to 10.6 days). In the first week of the test, the chips stained a bright red color. As the sampling and tests continued, the red color was less intense and it finally disappeared. Microscopically, the TTC stained protoplasm either had very faint red color, or stain was found as needle-shaped crystal-like material on the inner cell wall.

Since the early colonizing microorganisms (bacteria, Phycomycetes and *Fungi imperfecti*) live primarily on the free nutrients (starch, sugar, and nitrogen—Kaarik 1975), of the chips, it seems reasonable for the viability of the parenchyma cells and the development of the microorganisms to have a reciprocal relationship. That is, the more intact the parenchyma system, the less likely it would be to provide immediate nutrition to saprophytic microbes. Compression of the chips ruptured parenchyma cells and presumably increased the directly accessible amount of free nutrients on the surface of the chips but reduced the moisture content of the chips (from 80% to 65%, O.D. basis). Apparently, these combined effects account for the different microbial profiles between the compressed and noncompressed chips. Shortle and Cowling (1978) observed different microbial profiles and activities on sapwood blocks with and without living parenchyma cells. This may be explained by the conversion of starch, sugar, and other parenchyma protoplasm components to antimicrobial compounds (phenolic and tannic compounds) upon the death of parenchyma cells. This conversion process and the antimicrobial compounds may affect the development of microorganisms in injured or infected sapwood (Shain 1979; Wardell and Hart 1970). The noncompressed chips with relatively higher levels of intact parenchyma protoplasm were

less conducive to the development of bacteria but did permit growth of Phycomycetes. Compressed chips rewetted to the same moisture content as the non-compressed chips supported similar microbial development. This indicates that the development of the microorganisms is related directly to the moisture content of the chips. However, it is reasonable to assume that interactions of the accessibility of nutrients, parenchyma vitality, transformation of the parenchyma contents to antimicrobial compounds, and moisture content of the compressed chips could influence microbial development (as reflected in the isolation data).

It has been proposed that the initial heating in a wood chip pile is caused by respiration of living wood cells and the metabolic activities of bacteria (Feist et al. 1973; Springer and Hajny 1970). This effect was apparently accelerated in the compressed chips. The compressed chips, with their relatively high level of freed cell contents and early bacterial populations, showed a higher temperature profile at the initial stage of chip storage than noncompressed chips.

#### CONCLUSIONS

The compression of aspen chips at 27.6 MPa for 5 minutes reduced the viable stain area of the parenchyma cells by 60% and substantially reduced the half-life of the chip parenchyma.

The early microorganism profile was also affected by compression of the chips. The microorganisms most frequently isolated from the compressed chips were aerobic bacteria and Basidiomycetes as opposed to Phycomycetes and *Fungi imperfecti*, from the noncompressed chips.

Different levels of viable parenchyma and initial types of microorganisms were the most likely sources of the difference in the initial temperature profiles of the chip types. The compressed chip bales reached a maximum heating peak after one day of storage while the temperature of noncompressed chip pile simulators increased steadily.

The direct effect of compression on the viability of the parenchyma cells in green aspen chips was revealed in this study, but its effect on the long-term storage and resultant fuel potential of the wood chips requires further investigation.

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