

Moulds occurrence in woodchips

J. SOUČEK

Research Institute of Agricultural Engineering, Prague, Czech Republic

Abstract

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The research, whose results are presented, is aimed at determination of development of moulds number in wood chips under different storage temperatures. The experiments were carried out with the moisture of samples 65%, 22% and 1%. During the long-term storage the effect of water content in material on development of moulds can be recorded. The risks linked to mould occurrence can be considerably eliminated by reduction of water content.

Keywords: microbiology; hygienic risks; microscopical fibrous fungi; storage of biofuels

During the storage of wood chips an infestation by moulds can occur. The examination of concentration of microscopical fibrous fungi-moulds is not significant only in terms of storage possibilities (STUPAVSKÝ, HOLÝ 2010). These microscopical fibrous fungi-moulds are also mentioned as important allergens. From the hygienic point of view it is a serious problem, because these fungi-moulds produce the toxic substances – toxins. For quantification purposes the moulds are defined as the mesophilic aerobic fibrous microorganisms. On the mycological agar medium flat or fuzzy propagules or colonies are formed, often with coloured generative or spore-bearing structures.

ŠTENCL (2002) dealt intensively with the occurrence of moulds depending on water activity. He specified, among other things, the values of equilibrium moistures and water activities at samples of poplar wood chips in temperature interval 15 to 45°C. ZABEL and MORRELL (1992) dealt with influence of fungi and moulds on properties of wood mass. NEDĚLNÍK and MORAVCOVÁ (2006) describe the issue of occurrence of moulds in plant biomass in terms of risk of toxin formation. They assess the adverse effect of toxins on livestock nutrition.

In different kinds of tested samples of biomass, the mycotoxins were found in 93–100% of cases. PIECKOVÁ and JESENSKÁ (1999) deal in their work with the influence of moulds on human health.

The wood chips can be described as non-homogenous material of non-uniform composition, whose physical properties are changing in dependence on parameters of environment. In case when the wood chips are used for energy purposes, they are stocked for a long time period and therefore it is important to identify the various kinds of moulds occurring in them. At the different water content and different storage temperature, it is necessary to monitor the development of moulds number as the background data for application of effective means to elimination of health risks and microbiological degradation.

MATERIAL AND METHODS

The examined material was the wood chips of hazel. The hazel trees were freshly hewn and chopped for experimental purposes by means of chain saw (H 365XP; Husqvarna, Sweden) and a woodchip-

per (Pezzolato 110 Mb; Envie, Italy). Both these tools as well as a collecting vessel (50 l; Hornbach, Germany) in which the chopped mass was placed, were carefully rid of mechanical impurities and all their parts which can come into contact with material were cleaned by means of denatured alcohol. In this way the risk of material intoxication and subsequent distortion of results was minimized. The average size of wood chips was 4.46 mm.

The research was carried out at three different levels of moisture content. This different water content was achieved by the use of fresh sample (65%), partial drying (22%) and complete drying in laboratory hot air oven (at 60°C). During the handling with dried sample it came by autonomic accumulation of air humidity to its partial wetting (up to 1%). The samples treated in this way were poured into the small sterile vessels with the volume of 0.2 l, closed tightly and afterwards stocked under the constant temperatures –15°C (freezing box), 12°C (refrigerator), 25°C (thermostatic box 1) and 50°C (thermostatic box 2). The tightness of vessels was checked by comparison of sample weight in time of trial establishment and before realization of microbiological analysis.

The number of moulds was determined four times in the course of experiment – in time of its establishment and then after 7, 14 and 28 days.

Microbiological analyses were realised from homogeneous solution produced from wood chips. The starting suspension for microbiological analysis was prepared by shake-out of weighing of 10 g (60–70 pieces) wood chips sample up to 100 ml 0.1% pepton water. Further, tenfold dilution was chosen so that the resulting number of colonies grown in petri dishes was not higher than 150 (dilutions 10^{-4} , 10^{-5} and 10^{-6}). Inoculation was carried out so that 0.1 ml of inoculum was transferred by means of pipette on firm cultivation substrate and then spread on agar surface by a crooked glass rod. The prepared plates were incubated aerobically with lids turned upwards in thermostat at 25°C. The number of plates was read after 3–5 days of incubation. The cultivation was carried out on selective cultivation substrate with addition of an antibiotic. For preparation of cultivation substrate the dehydrated complete cultivation substrate and chloramphenicol agar with dichloran and Bengal red were used (both Viamar International, s.r.o., Prague, Czech Republic).

Total number of microorganisms (CFU – colony forming units) applied to one gram of sample was calculated according to the following formula:

$$N = \frac{\sum C}{(n_1 + n_2 \times d)} \quad (\text{CFU/g}) \quad (1)$$

where:

- $\sum C$ – sum of all colonies counted on selected plates
- n_1 – number of plates used to calculation from the first dilution
- n_2 – number of plates used to calculation from the second dilution
- d – factor of the first one for calculation of used dilution

Owing to a possibility of mutual comparison the results were applied to the quantity of dry matter in sample. The resulting value was calculated as:

$$P = N \times \frac{100}{s} \quad (\text{CFU/g d.m.}) \quad (2)$$

where:

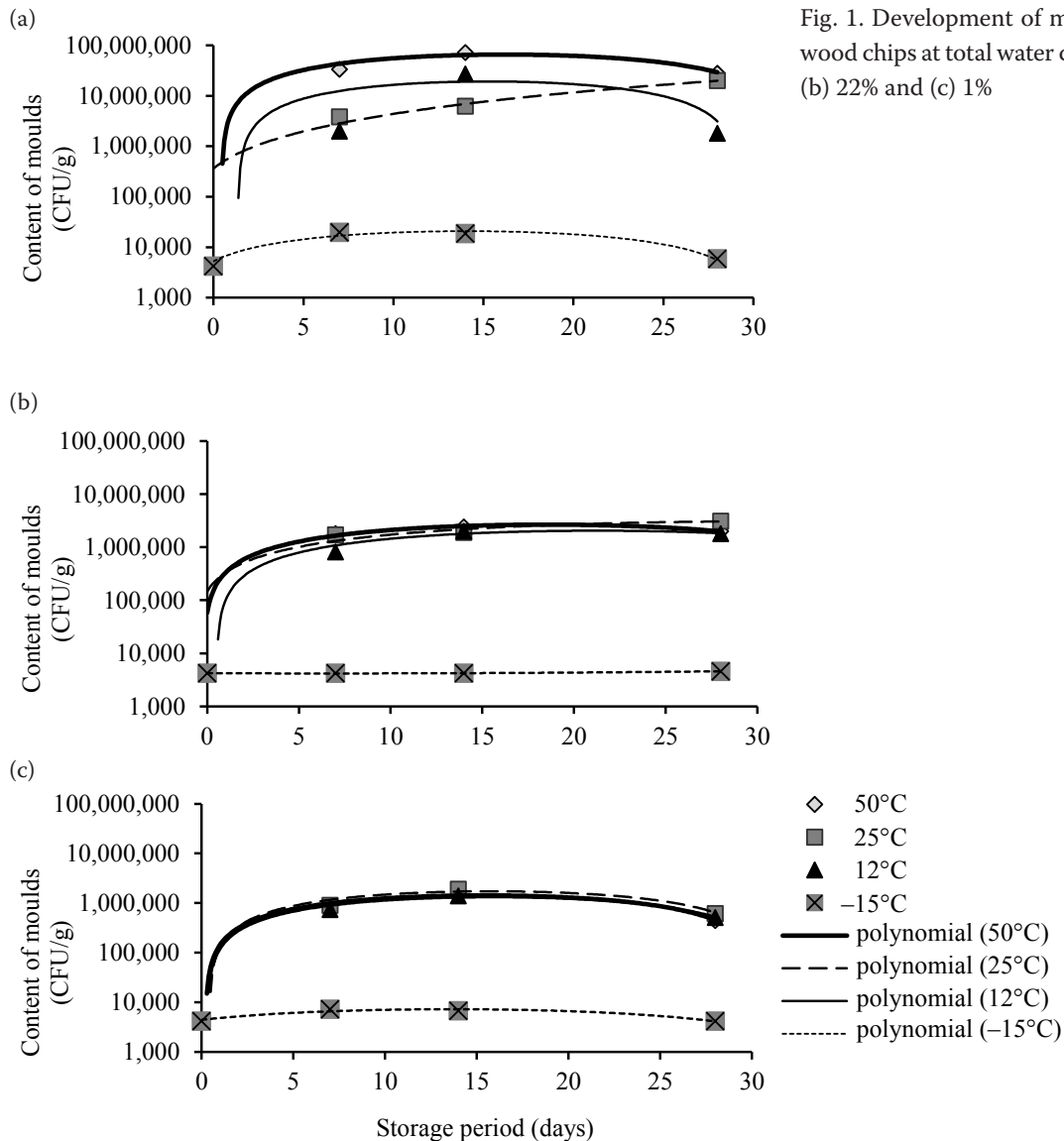
- P – resulting value of number of microorganisms
- N – sum of all colony-forming units (CFU/g)
- s – share of dry matter in sample (%)

RESULTS AND DISCUSSION

The quantity of moulds in stocked samples changed in the course of storage. The results are illustrated in Fig 1.

From the results presented in Fig. 1 is apparent that the water content in wood chips influences considerably the development of microorganisms during the storage and with it linked occurrence of risk factors actuating on ambient, in which the material is stored inclusive of hygienic risks. To these risks there are exposed both operating staff of storage facilities and personnel of technological processing lines.

The highest concentration of moulds was detected at the highest water content in material (65%). The values are approaching to 10^8 CFU/g d.m. Under high water content the effect of ambient temperature on the development of moulds number was the most evident. The highest concentration was recorded after 15 days of storage at the temperature of 50°C. The concentration of moulds at the temperatures of storage 50°C and 12°C at the beginning increased very quickly and after subsequent slowdown it came, approximately in the middle of experiment, to a gradual downturn. At the storage temperature of 25°C the initial increase was slower, but the gradual rise was recorded throughout the



period of experiment. This fact can be explained by species composition of moulds, for whose development the temperature of 25°C is optimal.

If the total water content in wood chips is on the level of 22%, the influence of temperature on the development of moulds number during the storage is less evident, but it is still very significant. At the temperature of 25°C the increase of moulds number is again gradual and continual. The concentration at the temperature of 12°C was lower in the entire course of experiment. The concentration of moulds at the temperature of 50°C in the first half of experiment duration was higher and afterwards its drop was recorded. At the temperature of storage -15°C no significant change in number of moulds was recorded.

In case of storage of dry wood chips (water content of 1%) the influence of temperature (with ex-

ception -15°C) on the course of moulds number in material is diminishing. At the favourable temperature of 25°C the number of CFU/g is still slightly higher, but the difference at the temperatures of 50°C and 12°C is insignificant.

At all temperatures of storage, the concentration of moulds in the second half of the experimental period had the downward trend.

The quantity of moulds in wood chips stocked at the temperature of -15°C did not change considerably in the course of experiment. At the samples with water content of 65% and 1% it came in the first stage to slight growth, however it is possible that it could come to this increase also by moderate involuntary intoxication during the establishment of the experiment. In spite of all realized measures it was not possible to avoid that. With regard to the used method, the differences were insignificant

from the microbiological point of view. The detected values of moulds content are consistent with results mentioned by ZABEL and MORRELL (1992).

In the samples of wood chips the moulds of the following genera were identified: *Cladosporium*, *Mucor*, *Penicillium*, *Alternaria*, *Botrytis*, *Aspergillus*, *Rhizopus*, *Fusarium*. The generic composition of moulds is practically identical with the results determined by NEDĚLNÍK and MORAVCOVÁ (2006). These researchers revealed in part of the examined samples the presence of secondarily produced mycotoxins, which can threaten the health of people and animals. However, the results of these authors confirm, in accordance with the conclusions of this article, that the development of formation of moulds and mycotoxins can be influenced considerably by modification of raw material properties and method of storage.

In comparison to the wood chips produced from conifers (ZABEL, MORRELL 1992) the content of moulds is higher owing to lower resin content. On the contrary, in comparison to soft wood of broad-leaved trees, which are often utilized for production of plant biomass, the content of moulds is lower. This is caused by structure and composition of wood of particular tree species.

CONCLUSION

From the obtained results arises that the long-term storage of wood chips with high water content is undesirable and this fact is in accordance with theoretical assumptions. With the exception of storage under frosty conditions, the number of moulds is, in comparison with dried wood chips, more than hundred times higher. In due proportion, there are also higher hygienic risks. Without the eventual use of preserving substances the bio-

degradation processes in stored material with high water content are certain to come through.

For the purpose of short-term storage (up to 20 days) it is not necessary to dry material to extremely low water content. At the water content around 20% in initial stage no significant differences were detected in regard to the content of moulds. However, during the long-term storage of wood chips it is desirable to store the material with water content as low as possible regardless of storage temperature owing to minimization of hygienic risks. From the practical point of view the water content in material during the storage can be recommended approximately at the level which is desirable for its further processing (it means 8–15% for production of briquettes and pellets and up to 17% for direct combustion). As a matter of course, the mentioned principles should be also applied for non-energetic utilization of wood chips.

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Corresponding author:

Ing. JIŘÍ SOUČEK, Ph.D., Research Institute of Agricultural Engineering, Drnovská 507,
161 01 Prague 6, Czech Republic
phone: + 420 233 022 214, e-mail: jiri.soucek@vuzt.cz
