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## Studies on Life Processes: Hydration of the Microenvironment and Fungal Growth

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Lignin and cellulose, the chief constituents of plant cell wall [1], have diverse physico-chemical properties. Lignin, a complex polymer, consists of phenyl propanoid units interlinked with non-hydrolysable carbon-carbon and ether bonds [2]. Cellulose, on the other hand, is a polymer of anhydrosugar units linked by glycosidic bonds in strongly hydrogen bonded parallel chains [3]. Cellulose in the plant cell wall is encrusted by lignin through covalent linkage [4]. In three-dimensional structure, the sheath-like presence of lignin provides a barrier to the biological hydrolysis of cellulose [1] thereby also reducing the availability of loosely held water in the vicinity [5,6] causing decreased water permeation [4] in the microenvironment. This barrier to hydration is broken down in nature by the preferential mineralization of lignin by white rot basidiomycetes [1] thus exposing hydrogen bondable sites in cellulose. Such a basidiomycete-lignocellulosic interaction provides a suitable system to study the hypothesis on aging and life processes [5] through the removal of hydrophobic encrusting of lignin and the creation of hydration sphere in the cellulose framework for the sustenance of life propagation processes. As a model to test this hypothesis, lignin in wheat straw was biodegraded by the white rot fungus *Coriolus versicolor*, to see whether it is possible to hydrolyse the hydrogen bondable moiety in the lignocellulosics for the introduction of water in a live system. Such a study expects to throw further light on the postulates of the hypothesis as affected by the microenvironment causing an increase in the hydration capacity in vital systems [5-8].

Composition of the culture medium, method of *C. versicolor* inoculum development, preparation of wheat straw as substrate, inoculation procedure and incubation conditions were the same as reported earlier [9]. During the solid state fermentation of 7, 14, 21 and 28 days, losses on account of biodegradation of the substrate in the contents of lignin and organic matter were, respectively, determined according to Khudayakova [10] and Zadrazil *et al.* [11]. Water retention capacity (WRC) of the wheat straw fermented for various periods (designated as live fermented biomass – LFB) was determined by hydration to saturation, immediately after removal from incubation at 25°, by soaking in water for 2 hrs. The excess water was removed by filtration and the hydrated straw, after weighing, was allowed to dehydrate at 25° for 24, 48 and 72 hrs and the dehydrated straw weighed again. Difference in the two weights expressed on dry weight basis, represented percentage WRC of LFB. Portions of the wheat straw fermented during the corresponding periods, in another set, were oven-dried at 105° for 24 hrs. These oven-dried portions (designated as killed fermented biomass-KFB) were hydrated and dehydrated to determine their WRC in the same manner as done for LFB. Sodium and potassium were determined flame photometrically in the washed fermented wheat straw, 5 g of which was ashed at 540°, dissolved in 10 ml of 6N HCl, filtered and made up to 50 ml.

The fermented biomass as harvested (LFB) may be regarded as a living system since the stationary phase in the biodegradation of both lignin and organic matter had not been reached upto the 28-day incubation period under study (Fig.1). The same biomass upon oven-heating at 105° for 24 hrs (KFB), on the other hand, may be regarded as a killed system. The WRC for both LFB and KFB in the wheat straw biodegraded for 7, 14, 21 and 28 days after 72 hrs and during the progressive stages of 24, 48 and 72 hrs is presented, respectively, in Fig. 1 and Table 1. As against the WRC of 5.4% after 72 hrs in the non-fermented wheat straw, the corresponding values in the LFB were 6.5, 6.7, 10.2 and 16.4% during the fermentation periods of, 7, 14, 21 and 28 days, respectively. It may be noted that the WRC increase corresponded with the increase in lignin degradation as the fermentation period

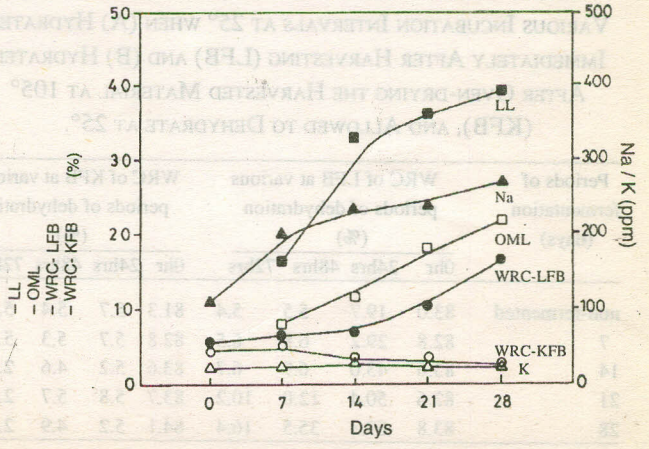


Fig. 1. Wheat straw-*Coriolus versicolor* fermentation at 25° for various periods: loss of lignin (LL-■-) and organic matter (OML-□-); water retention capacity upon dehydration for 72 hrs at 25° in live fermenting biomass (WRC-LFB-●-) and in killed fermented biomass (WRC-KFB-○-); and the fermentation-in system levels of Na/K (Na-▲-) and (K -△-).

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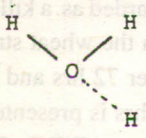
advanced (Fig. 1). A likely explanation to the increase in WRC can be: (a) with the increased removal of hydrophobic lignin, more hydrophilic cellulose became exposed in the system [12]; (b) a living system was activated, which continued to increase in mass and activity as the fermentation stages progressed; and (c) as a result of biodegradation, the degraded lignins, which have been shown to contain higher proportions of ionisable groups, such as carboxyl [13], may contribute to the formation of low molecular weight hydrogen-bondable and water-soluble lignin units. In the KFB, the WRC was observed to be 5.2, 2.3, 2.2 and 2.2% during the fermentation periods of, 7, 14, 21 and 28 days respectively, as against 5.4% in the non-fermented wheat straw (control) after 72 hrs. It is evident from these observations that WRC decreased appreciably in the KFB, indicating the adverse effect of killing and the vital nature of water binding in the hydrophilic biopolymers/living systems. It is possible to explain the mechanism that controls the WRC in the living and dead organic systems by considering the fermentation mass as a complex. This makes it possible to hydrate the microenvironment of this particular bond, forming a transition couple such as the following scheme.

Lignin - Cellulose  Lignin-H + Cellulose-OH



The organism frees the cellulose but not before it has degraded the lignin, which loses at least one carbon atom. The site at which the hydrolytic attack occurs is now hydrogen bondable and hence gains in the WRC. On oven-drying this living system, the very microenvironment, which was opened up by hydration, gets dehydrated. The loss of a molecule of water reduces the hydrogen bondability and hence the lower values of WRC of the killed system (KFB).

In order to study the possible role of metal ions in WRC, the "in-system" K and Na levels were determined, at various fermentation stages, after thorough washing of the LFB. K level in the LFB remained almost unchanged; Na level, on the other hand, was observed to increase as the fermentation period progressed. The increase in Na was also noted to have a trend comparable with the WRC in the LFB (Fig. 1). From these observations it may be concluded that Na ion becomes an integral part of the fungus-substrate system under study. Na has, thus, a vital role corresponding well with increased bioacitivity while K shows no such correlation and perhaps is not needed in quantities more than those noted. It is possible that the Na ion by becoming an integral part of the fermenting system contributes to its turgidity and thus to a higher uptake and retention of water.

***Key words:*** Hydration, Microenvironment, Fungal growth.

TABLE 1. WATER RETENTION CAPACITY (WRC) OF WHEAT STRAW-*CORIOLUS VERSICOLOR* FERMENTATION SYSTEM AT VARIOUS INCUBATION INTERVALS AT 25° WHEN (A) HYDRATED IMMEDIATELY AFTER HARVESTING (LFB) AND (B) HYDRATED AFTER OVEN-DRYING THE HARVESTED MATERIAL AT 105° (KFB), AND ALLOWED TO DEHYDRATE AT 25°.

| Periods of fermentation (days) | WRC of LFB at various periods of dehydration (%) | | | | WRC of KFB at various periods of dehydration (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0hr | 24hrs | 48hrs | 72hrs | 0hr | 24hrs | 48hrs | 72hrs |
| non-fermented | 83.0 | 19.7 | 5.5 | 5.4 | 81.3 | 5.7 | 5.4 | 5.4 |
| 7 | 82.8 | 29.2 | 6.8 | 6.5 | 82.8 | 5.7 | 5.3 | 5.2 |
| 14 | 83.6 | 43.0 | 6.9 | 6.7 | 83.6 | 5.2 | 4.6 | 2.3 |
| 21 | 82.6 | 50.4 | 12.0 | 10.2 | 83.7 | 5.8 | 5.7 | 2.2 |
| 28 | 83.8 | 69.3 | 35.5 | 16.4 | 84.1 | 5.2 | 4.9 | 2.2 |

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