

OXYGEN CONSUMPTION BY A WET GRAIN ECOSYSTEM IN HERMETIC SILOS AT VARIOUS WATER ACTIVITIES

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Abstract

General rules of microbiological stabilization of wet grains by the use of controlled atmospheres are now well established for cereal grains and feasibility depends largely on water activity. Nevertheless, the advisability of setting up a strict anoxia at the beginning of storage, whatever the process employed for oxygen elimination, or the possibility of letting the ecosystem generate anoxia by natural consumption, remains an open question.

Recent results obtained in experimental micro-silos showed that the rate of oxygen decreased as a function of water activity of the grain. At high water activity values (i.e., $a_w = 0.90$), the amount of oxygen contained in the initial intergranular atmosphere was sufficient to allow a limited microbial evolution due to microaerophilic species. At $a_w < 0.85$, oxygen progressively disappeared without measurable microbial changes. Considering the relationship between the quantities of CO_2 produced and of O_2 absorbed by the ecosystem, it was shown that, as expected, the ratio (CO_2 produced to O_2 absorbed) was constant (close to 0.65) for grains with $a_w < 0.85$. At higher a_w levels, some carbon dioxide was produced by fermentation and the ratio increased up to 0.90 at $a_w = 0.89$, and 1.5 at $a_w = 0.94$. These data suggest a possible way to estimate rapidly the microbiological state of stored grains but they also indicate that further research is needed to understand fully the phenomenon of CO_2 adsorption by grains.

The possibility of opening hermetic silos for grain utilization in the course of storage implies practical application of these results.

Introduction

Grain storage in airtight structures is probably a very ancient method for preserving dry grains from insect attack; however, interest in hermetic storage of wet grains is more recent. The technique seems to be known empirically in Europe from the middle of the 17th century, but as far as we know, scientific experiments began

in this field only after the second world war. Its use is now well established in France, for storing maize with very high moisture content, for use as feed.

Experiments carried out on grains stored hermetically with moisture content (m.c.) between 16 and 22 %, are reported as being successful in preventing deterioration of the treated commodity (Hyde and Oxley, 1960 ; Shejbal, 1979 ; Richard-Molard et al., 1984), but other reports also indicate grain deterioration, mainly due to unfavourable evolution of grain microflora.

However, it seems now well established that an efficient protective effect can be obtained against molds if damp grain is stored, even for a long time, under atmospheres with very low oxygen content (Gonen and Calderon, 1968 ; Clarke and Hill, 1971).

In most situations low-oxygen conditions are produced by the respiration of the grain ecosystem itself but it has often been stated that when moisture content is relatively low, about 17 % wet basis (w.b.) for wheat for example, the natural reduction of oxygen becomes too slow and that insects and molds can continue to develop slowly (Hyde, 1973 ; Shejbal and De Boislabert, 1982).

Therefore, the advisability of creating oxygen free conditions at the beginning of storage, whatever the technique employed, or the possibility of letting the ecosystem generate anoxia by natural consumption of oxygen in the intergranular atmosphere remains an open question. A closely related question concerns the possible deterioration of grains due to oxygen penetration if bins are opened at intervals for grain removal.

Results presented in this paper were obtained in strictly hermetic micro-silos on a laboratory scale. They enabled determination of the rate of oxygen decrease and carbon dioxide increase, depending on water activity of the grain. They were used to discuss the opportunity of creating artificial low-oxygen atmospheres in wet grain storage under modified atmospheres.

1. Materials and methods

1.1. Micro-silos and measuring equipment

All experiments were conducted on paddy stored in micro-silos of 10 l capacity, the airtightness of which were checked by filling with pure nitrogen and measuring the level of oxygen during about ten days. Evolution of the internal atmospheric composition was studied by measurements of O_2 (magnetodynamic O_2 -meter) and CO_2 (IR absorption, BINOS CO_2 -meter) at intervals depending on the water activity of the grain.

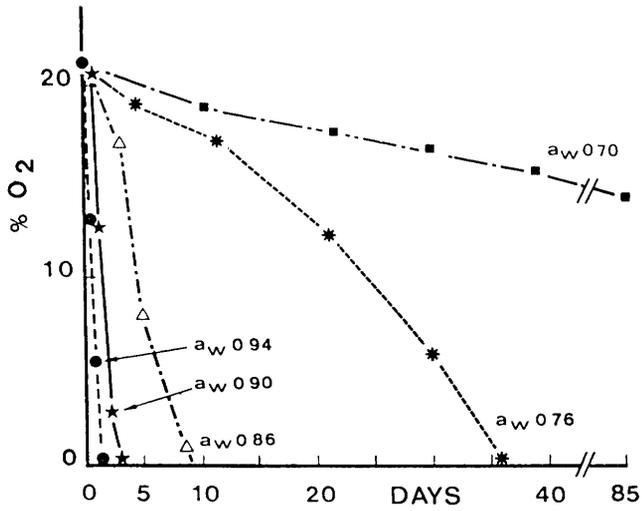


Figure 1 : Oxygen consumption by grain ecosystem as a function of water activity.

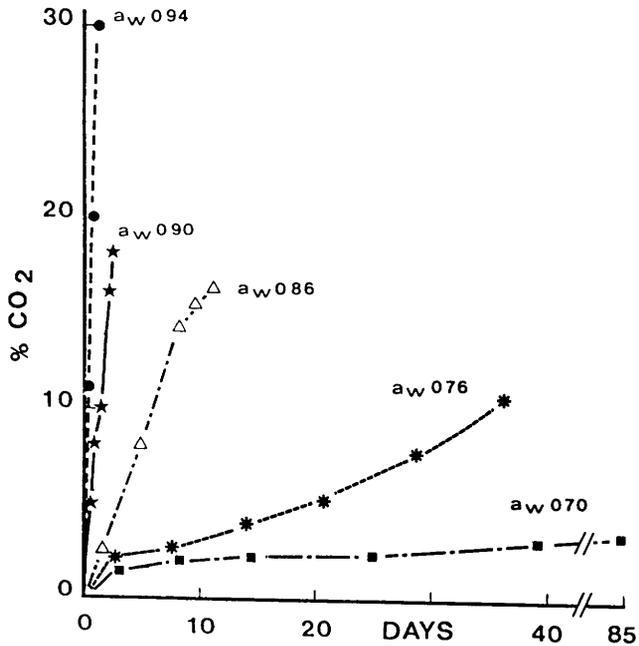


Figure 2 : Carbon dioxide production in relation to water activity.

1.2. Moisture content

Paddy was initially at about 14 % m.c. and was rehumidified to obtain various water activities, by addition of distilled water according to the sorption isotherms. After a period of equilibration of about 4 days at 4°C, the micro-silos were completely filled with about 6,5 kg of paddy, sealed and placed in a temperature controlled room at 24°C. In all experiments, paddy was artificially inoculated during rehumidification by a toxigenic strain of Aspergillus flavus to test the possible biosynthesis of mycotoxin under the given experimental conditions.

1.3. Microbiological methods

After each opening of the silos, bacteria, yeasts and molds were evaluated by classical dilution methods, with plate count agar for mesophilic aerobes, rogosa agar for Lactobacillus spp., and malt agar for yeasts and molds. It is well known that for most fungi, sporulation is always considerably reduced in an atmosphere of limited O₂ concentration. So for every condition, mycelial growth of fungi was tested using ergosterol determination by HPLC (Cahagner et al., 1983). Fungal ergosterol was extracted with methanol, saponified with KOH, reextracted with petroleum ether, purified on silica gel and analysed by HPLC using a 5 µ-spherisorb column. Aflatoxin B₁ was also determined by an HPLC procedure using a fluorimetric detector.

2. Results and discussion

2.1. Changes in the intergranular atmospheric composition

Figure 1 shows that the rate of oxygen decrease at the beginning of the experimental storage under airtight conditions depends largely on water activity of the grain. With an a_w of 0.94 (i.e. a moisture content of about 24 % w.b. at 24°C) complete anoxia was obtained within only a few hours. At a_w 0.89, the oxygen level fell to zero after about 3 days but with m.c.s. lower than 16.5 % (a_w 0.86) it took a long time to reach complete anaerobic conditions. In fact at an a_w of 0.70, only a slight decrease of oxygen level was observed after three months.

Figure 2 shows the increase in CO₂ concentrations observed at the same time. As expected, the rate of CO₂ production was closely related to oxygen consumption and very high CO₂ concentrations were obtained in micro-silos in which m.c. exceeded 19-20 %, that is to say when fermentation began to take place.

At a_w 0.94, lactic acid bacteria grew very rapidly and reached a number of^w about 10⁸/g within 20 hours. Lactobacillus plantarum and two heterofermentative species not yet identified were isolated. At the same time, "yeast" counts suggested a possibly significant growth of Candida variabilis (Lind.) Berkout, and Aureobasidium pullulans (De Bary) Arnaud, a yeast-like fungus which was counted on the same plates (Figure 3).

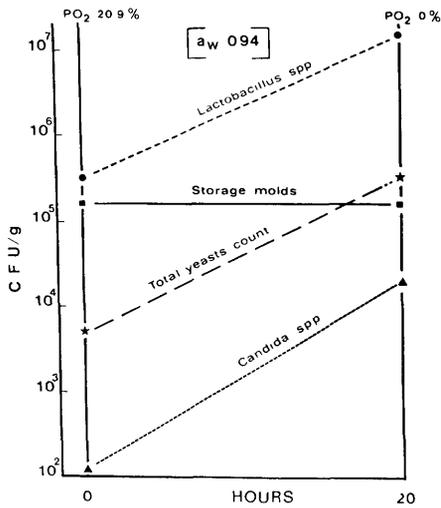


Figure 3 : Apparent microbial development in paddy during evolution of natural anoxia at a_w 0.94.

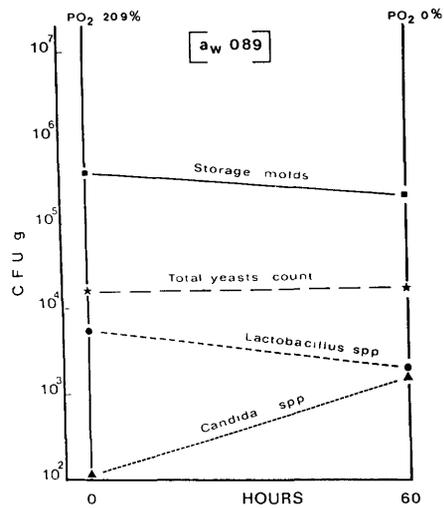


Figure 4 : Apparent microbial development in paddy during evolution of natural anoxia at a_w 0.89.

From the above figures, it appears that lactobacilli were indifferent to oxygen as long as its partial pressure remained low. So it can be assumed that the oxygen content in the micro-silos was sufficient to allow a limited growth of yeast and yeast-like fungi. At $a_w = 0.89$, growth of lactobacilli seemed to be impossible (Figure 4) and yeasts became dominant during the first three days. It should be noted that although a clear sour odor was detected in grain at a_w 0.94, the organoleptic properties of paddy were not appreciably modified during the period of natural O_2 elimination at a_w 0.89. At water activities lower than 0.85, oxygen progressively disappeared without any measurable microbiological change. Neither could mold growth or mycotoxin formation be detected in paddy, and ergosterol contents remained perfectly constant whatever the moisture content of the grain and the time require to reach the anaerobic state. Our conclusion from these experiments was that as long as the available oxygen was restricted to the initial content in the intergranular atmosphere, no significant changes could be attributed to this intergranular oxygen for paddy stored in airtight conditions.

2.2. Apparent respiratory quotients (R.Q.)

Few studies have been made on the effect of moisture content on the apparent cumulative respiratory quotients of grain, i.e. the relationship between the quantities of CO_2 produced and of O_2 absorbed by the ecosystem. Results from Hyde and Oxley (1960) showed that for barley, stored under airtight conditions in small metallic bins at moisture contents of about 18 to 24 %, this relation was quite linear, giving an apparent R.Q. of about 0.60. In our experiments, the values

of carbon dioxide produced plotted against the oxygen consumed gave very similar results provided that the a_w remained below 0.85 - 0.87. At higher a_w levels, as shown in figure 5, experimental data demonstrated a linear relationship between O_2 and CO_2 but led to apparent R.Q. consistently higher, near to 0.90 at a_w 0.89 and 1.5 at a_w 0.94.

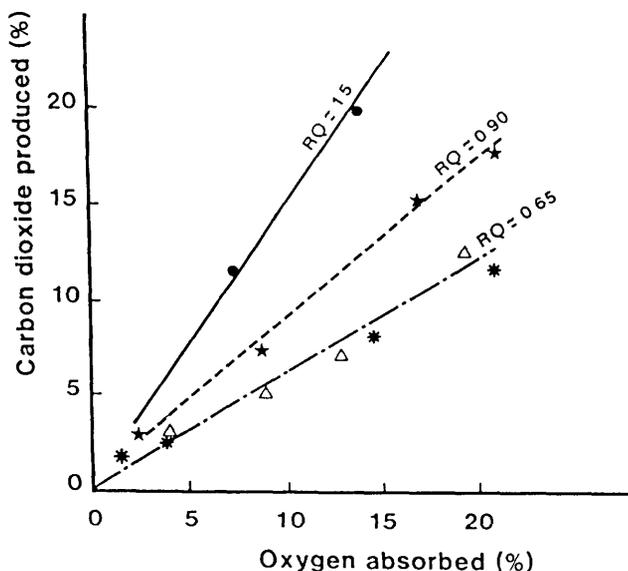


Figure 5 : Relationship between CO_2 produced and O_2 adsorbed at various a_w during hermetic storage of paddy.

The apparent excessive CO_2 production at moisture contents higher than 19 % was due to fermentation by lactic acid bacteria and possibly by yeasts as suggested in figures 3 and 4.

According to present knowledge, one can assume that at a_w lower than 0.85, no CO_2 can be produced by fermentation and that all the intergranular oxygen is converted to carbon dioxide by respiration of xerotolerant molds, insects if present, and the grain itself. Considering that about 40 % of the total grain bulk volume was occupied by intergranular atmosphere, it can be calculated that the micro-silos contained about one g of oxygen, allowing for respiration of no more than one g of dry matter if carbohydrates or lipids are mainly consumed. So dry matter losses by consumption of initial oxygen cannot exceed 0.02 % provided no fermentation occurs. In order to simulate openings of the silos, the same experiments were repeated three times with the same grains, silos being flushed with compressed air until the oxygen level returned to 20.9 %. After the micro-silos were resealed the changes in O_2 and CO_2 content were quite similar to

those previously described and so we considered that at least for cereal grains, there is no real interest in creating artificial anaerobic conditions at the beginning of airtight storage.

Nevertheless, further research is needed to determine the lower limit of moisture content allowing fermentation processes, by yeasts and yeast-like fungi in grains of intermediate m.c.

2.3. Carbon dioxide adsorption

For non fermenting grain (i.e. at a_w lower than 0.90) the apparent deficit in CO_2 production compared to O_2 absorbed can be explained by adsorption of a part of the CO_2 produced on the grains.

From our experimental results, assuming that all the CO_2 lacking is adsorbed on the grain, adsorption coefficients can be calculated as shown in Table 1.

Table 1 : Adsorption of CO_2 on paddy at various a_w .

A_w	Total CO_2 (g)	CO_2 in the gas phase (g)	CO_2 adsorbed (g)	Adsorption Coefficient (ml/g)
0.76		0.72	0.73	0.057
0.85	1.44	1.10	0.34	0.038
0.90		1.23	0.21	0.017
0.70				0.17

Calculated values obtained in our experimental conditions are about five times lower than those published Yamamoto and Mitsuda (1980) in their study on CO_2 adsorption by rice. We observed also an increase in adsorption capacity when a_w decreased (with dry grain (a_w 0.70) the adsorption was determined by putting the grain in pure CO_2 atmosphere during 36 hours and measuring the residual partial pressure of CO_2).

On the other hand, if the coefficients proposed by Yamamoto and Mitsuda (1980) (0.3 -0.4 ml/g) are used, the balance O_2/CO_2 does not correspond with our results. These results suggest that CO_2 adsorption depends not only on m.c. but also on the physical state of the grain and, clearly better knowledge in this field would be of major interest for further clarifying microbial behaviour under airtight conditions.

3. Conclusion

The experimental data presented in this paper show that for grains at a_w below 0.90 it is not really necessary to create artificial anaerobic conditions at the beginning of airtight storage or after opening the silos. For grains with higher moisture contents

or for airtight storage over very long periods, facultative anaerobes (bacteria and yeasts) can develop and produce off-odors which can be detrimental if the grain is not to be utilized as animal feed.

Further studies are necessary to determine the exact role of oxygen and carbon dioxide on the growth of the particular microorganisms discussed.

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