USE OF AN AUTOMATIC ELECTROLYTIC RESPIROMETER TO STUDY RESPIRATION OF STORED GRAIN.

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ABSTRACT

An electrolytic respirometer system (MP-686, Maynard Projects, Cambridge), has been used to measure the respiration of cereal grain stored at 15 - 25 °C with 0.70 - 0.95 water activity (a_w). Respiration of wheat grain cv. Avalon increased with a_w and temperature, markedly at a_w above 0.85. Visible moulding was evident in some samples stored at 0.90 a_w and above, depending on temperature. Respiratory quotients were generally close to 1.0. Over 7 d, there was a linear increase in oxygen consumption above a_w of approximately 0.90; below this, respiration increased exponentially with time. This may be because the relative contributions made by grain and fungi to the total respiration measured are differentially affected by differences in environmental conditions.

INTRODUCTION

Moulding of grain occurs when sufficient water is available for fungal growth, i.e. with a_w > 0.65. The presence of ‘visible mould’ has been widely accepted as indicating the time after which grain can no longer be safely stored (Kreyger, 1972). However, but this is unreliable (Seitz et al., 1982a) as it is not known whether grain quality is affected before this stage. Measurement of grain respiration could be used to quantify microbial activity before moulding becomes visible.

Respiration of stored grain is due to microbial respiration, particularly from fungi, (Norman et al., 1941; Christensen, 1955) and respiration of the grain itself (Hummel et al., 1954). The relative contributions of these two components remains controversial (Pomeranz, 1974). Larmour et al. (1935) and Hummel et al. (1954) recorded only small amounts of respiration from mould-free wheat grain at 12 - 35% water content, while respiration from fungi was no more than 10% of the total respiration of sterilised maize grain (Woodstock and Combs, 1965). With maize at 22 - 27% water content, respiration by the surface microflora was much less than that of the grain itself (Seitz et al., 1982b). Separating the two components could allow quantification of the fungal biomass from measurements of total respiration.

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Grain respiration is largely governed by water availability and temperature and, to a lesser extent, by oxygen concentration, microbial contamination, kernel size, mechanical damage of kernels, the conditions and length of previous storage and the degree of mite and insect infestation (Bailey, 1940; Milner and Geddes, 1945; Steele et al., 1969). Respiration appears to increase exponentially with temperature and water content, i.e. linearly with water activity (Bailey, 1940; Srour, 1988).

Aerobic respiration of grain involves the oxidation of carbohydrates and results in the release of heat energy, water and 14.7 g CO₂ per kg of substrate for every 1% loss of dry matter (Steele et al., 1969). This assumes respiratory quotients (RQ) of 1.0. Lipid and protein metabolism (where RQ < 1.0) and anaerobic fermentation (RQ > 1.0) have been considered negligible in stored cereal grain respiration, although important in oilseeds (Saul and Lind, 1958; Steele et al., 1969; White et al., 1982). Thus, theoretical losses in dry matter can be calculated for given amounts of respiration, and predictions can be made of the storage periods that will give a predetermined dry matter loss under different environmental conditions.

A range of methods have been used to measure respiration but some are destructive, which prohibits continuous monitoring. Sealed units have been used, in which oxygen is not replaced and CO₂ may accumulate (Pomeranz, 1974) but this may be sufficient to inhibit aerobic respiration of microorganisms, so that intervals between sampling should be short.

Recently, an automatic electrolytic respirometer has been designed which continuously records the production of O₂ by electrolysis to replace respired CO₂ which has been absorbed in alkali (Tribe and Maynard, 1989). Among its advantages over other systems are that up to 128 samples can be assessed in one experiment; O₂ is generated in the system in response to its uptake; CO₂ does not accumulate to inhibit respiration, and the system remains completely enclosed from the external environment. This instrument was used to study the respiration of naturally contaminated grain under a range of a₁ and temperature conditions to determine the rate and pattern of grain respiration. Respiration was related to kernel infection by the predominant fungi.

MATERIALS AND METHODS

Description and Assembly of Respirometer

Grain samples and a vessel of sodium hydroxide to absorb CO₂, were enclosed in a tube maintained at constant temperature. This is connected, through a U-tube, to a compensator unit of approximately equal air volume. Each U-tube contained electrolyte, a platinum anode and a copper cathode. O₂ uptake by the grain decreases the pressure in the sample unit, causing the electrolyte level to rise under pressure from air in the compensator unit and make contact with the anode. Current flows, generating O₂ at the anode, until pressure is equalised when contact at the anode is broken and current can no longer flow, and the cycle repeats as respiration continues. The currents flowing were continuously recorded and converted to the volume of O₂ consumed by the computer.

Respirometer units were assembled as shown in Fig. 1. The glass tubes contained 25 g subsamples of treated grain and bijou tubes, containing 5 ml 2M NaOH, were placed on top of the sample. Sixteen tubes were placed in each of up to 8 stainless steel racks and electrodes were connected via connector strips and a four channel multiplexed ammeter inside an Electronic Control Unit (ECU) to a BBC Master series microcomputer with monochrome monitor and dual disk drive. The
computer software included programs to run the data sampler and print and plot results. Racks were immersed in water baths for 30 min to allow temperature equilibration, and lead collars were placed around each unit to prevent the compensator bottles from floating. Suba-seal caps were closed and the ECU switched on to supply current to each respirometer unit, data was sampled at 15 s intervals, and recorded to disk every hour. Experiments included at least three replicate units of each treatment and were repeated at least once. During preparation of CO₂ absorption tubes, controls were prepared to determine the absorption of atmospheric CO₂.

Figure 1

Schematic Representation of a Single Respirometer Unit.

1) Glass tube 280 mm x 27 mm; 2) Bottom bung, 3) Top bung, 4) Alkali vessel, 5) Electrolyte, 6) Copper cathode, 7) Platinum anode, 8a and b) Glass tubes, 9a and b) Suba seal caps through which the electrode wires are threaded, 10) Compensator Bottle, 11) Grain sample.
Electrolyte, made up of 625 g CuSO₄·5H₂O / 1 M H₂SO₄ to form a super saturated solution, was distributed to give 8-9 ml in each electrolysis cell. When the electrolysis cell was assembled, levels of electrolyte were adjusted so that the anode tip was slightly above the meniscus of the electrolyte. Joints made by rubber bungs with glass tubes and compensator bottles were made watertight by sealing with silicone rubber compound. Temperatures of 15, 20 and 25 °C were maintained with thermostatically controlled aquarium heaters (Visitherm) in water baths (0.76 x 0.365 x 0.3 m glass aquaria) and tanks were insulated with panels of expanded polystyrene.

Dismantling

After 165 h incubation, units were dismantled and CO₂ absorbed by the NaOH was quantified by titration. Grain removed from each unit was assessed for visible moulding, and samples were plated to determine the predominant fungi.

Titration
NaOH was diluted tenfold with distilled deionised water (DDW) and titrated against standardised 0.2 M HCl. The volumes of acid required to attain 8.3 and 4.0 pH as indicated by colour changes in phenolphthalein (0.5% phenolphthalein (BDH) in 95% ethanol) and screened methyl orange (BDH '3046') indicators, respectively, were recorded. Volumes of CO₂ absorbed by the NaOH solution were calculated from the equation:

\[ \text{ml CO}_2 \text{ (dry, NTP)} = (V_2 - V_1 - (C_2 - C_1)) \times 22.44 \]

where \( V_1 = \text{ml HCl to pH 8.3 (sample)} \), \( V_2 = \text{ml HCl to pH 4.0 (sample)} \), \( C_1 = \text{ml HCl to pH 8.3 (control)} \), \( C_2 = \text{ml HCl to pH 4.0 (control)} \). Respiratory quotients (RQ) were calculated using the formula:

\[ \text{RQ} = \frac{\text{total ml CO}_2 \text{ produced}}{\text{total ml O}_2 \text{ consumed}}. \]

Direct Plating
Ten grains from each tube were plated directly onto each of three Malt Glycerol Agar plates (MGA) (g/l sterile DDW: Malt Extract (Oxoid L39), 20; Agar (Oxoid L13), 20; adjusted with glycerol to 0.95, 0.90, 0.85 and 0.80 \( a_w \) (Dallyn and Fox, 1980); Autoclaved at 121 °C / 15 min.). Plates were incubated at the same temperature as used for the sample during the respiration study and fungi counted and identified after 7 -28 d. Standard texts (Samson et al., 1981; von Arx, 1970) were used to classify fungi to genus.

The Effect of \( a_w \) and Temperature on the Respiration of Wheat Grain

The respiration of 0 - 1 year old wheat grain cv. Avalon was studied at a range of \( a_w \) from 0.65 (14.0% water content) to 0.95 (27.0% water content), and at 15, 20 and 25°C. \( a_w \)'s were determined at 25°C (Humidat IC II (Novasina AG, Switzerland)). Water content (% wet basis) was calculated using the formula:

\[ \text{WC} = \frac{\text{WW} - \text{DW}}{\text{WW}} \times 100; \text{where WC} = \text{water content}; \text{WW} = \text{Wet weight (g)}; \text{DW} = \text{Dry weight (by oven-drying 5 or 10 g whole grains at 105 °C for 16 h)}. \]

RESULTS AND DISCUSSION

Visible Moulding and Fungal Colonisation

There was no evidence of visible mould after 7 d incubation at 15°C. By contrast, samples incubated at 20°C with 0.95 \( a_w \) and at 25°C with 0.95 and 0.90 \( a_w \) had moulded, with more mould visible at 25°C than 20°C.
Two types of respiration curve were obtained (Fig. 2). With $a_w$ of 0.85 and below, the volume of oxygen consumed by the sample increased in a manner suggesting an exponential phase in oxygen consumption. However, with $> 0.90$ $a_w$, the volume of oxygen consumed increased linearly with time. A possible explanation for this may be that the balance between the contributions of the two components differs. Above 0.90 $a_w$, the seed may be rapidly invaded by fungi (as indicated by visible moulding), killing the embryo, so that there is no contribution to the total respiration from the grain itself. However below 0.90 $a_w$, the embryo may remain active, with little or no fungal invasion of the seed. If so, the data recorded at 0.95 $a_w$ (Fig. 2.) may come only from fungal respiration, and at 0.85 $a_w$, the respiration may be a result of both components, with that of the grain being dominant. Alternatively, the linear increase in O$_2$ consumption at high $a_w$ may represent only part of an exponential curve with the initial phase having occurred very rapidly because of the high $a_w$ of the sample, and not being detectable in experiments completed so far.

Fig. 3 suggests that there is an exponential increase in O$_2$ consumption with increasing $a_w$, a linear increase with water content, and that respiration rate also increased with increasing temperature. These results agree with previous findings (Bailey, 1940; Srour, 1988). Respiratory quotients were generally close to 1.0, suggesting that carbohydrate utilisation formed the predominant part of respiration.
Field fungi (Alternaria and Cladosporium spp.) were isolated only from samples incubated at high $a_w$ (Fig. 4.) although in smaller numbers than could be isolated from control (unincubated) samples on the same media and temperature. Storage fungi (Aspergillus, Eurotium and Penicillium spp.) increased in number during incubation under most $a_w$ / temperature conditions. Aspergillus spp. were isolated in greatest numbers from samples incubated at 25°C and 0.90 $a_w$. Penicillium spp. were most numerous at $\geq 0.90$ $a_w$ and Eurotium spp. at 0.85 $a_w$ (with incubation temperatures of 15 and 25°C) and 0.90 $a_w$ (20°C incubation).
Figure 4.
Changes in fungal infection of kernels after 7 d storage.

Water Activity

<table>
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<tr>
<th>Temperature</th>
<th>0.80</th>
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<td>15°C</td>
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<td>25°C</td>
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Fungus:
- Alternaria
- Aspergillus
- Cladosporium
- Eurotium
- Penicillum
CONCLUSIONS

The pattern of respiration recorded by the respirometer is a function of the respiration of the grain itself and also of fungi as they colonise the internal and external parts of the grain. Optimum environmental conditions are different for both components and also for different fungal taxa.

Only the main trends in respiration should be considered, and it is still necessary to fit equations to the curves to describe the relationships between respiration rate, water activity and temperature. Temperature recording and logging hardware is currently being developed so that temperatures within the respirometer units and water baths can be accurately recorded.

REFERENCES


Kreyger, J. (1972) Drying and storing grains, seeds and pulses in temperate climates. IBVL publication 205, Wageningen, Holland.


On a utilisé un respiromètre électrolytique (MP-686, Maynard Projects, Cambridge), couplé à un dispositif de traitement logarithmique de la température, pour mesurer la respiration de grains de céréales stockés de 10 à 30 °C entre 0,65 et 0,98 (a_w). La respiration de semence de froment cv. Avalon a augmenté avec la température (a_w). Avec plus de 0,92 (a_w) la consommation d’oxygène sur plus de 7 jours a augmenté linéairement avec le temps. Avec moins d’eau, la respiration a augmenté exponentiellement avec le temps. Les quotients respiratoires étaient partout proches de 1,0. L’irradiation gamma et les traitements antibiotiques ont été utilisés pour inhiber la croissance d’une partie ou de la totalité de la microflore, dans le but de distinguer la respiration bactérienne et fongique de celle du grain, dans la respiration totale. De petites doses d’irradiation ont tué la plus grande partie de la microflore et augmenté le pourcentage de germination du froment mais n’ont pas affecté la respiration totale. Au contraire, certains traitements aux antibiotiques ont stimulé la respiration bien que les populations de microflore aient diminué.