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YEAST INTRACELLULAR WATER DETERMINATION BY THERMOGRAVIMETRY

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Abstract

The intracellular water content of a microorganism is an important parameter which is a determinant factor of its physiological properties. It is usually measured by complex and time consuming procedures. Thermogravimetry using infrared balance has been used for this purpose, through the identification of different drying steps occurring during the analysis. This work employs the same method with much smaller samples, using conventional thermogravimetric equipment in a simpler and faster way than other conventional procedures.

Commercial yeast (*Saccharomyces cerevisiae*) washed samples are analyzed in isothermal procedures which are run in about 30 min. The drying rate curve, when plotted as a function of the residual mass of the cells, allows the identification of the step where the intracellular water is lost and the determination of its content. The obtained values, on extracellular water free basis, are in the range of 65 to 69% and agree with those measured by other techniques.

Keywords: drying, intracellular water, *Saccharomyces cerevisiae*, TG

Introduction

The yeast cell boundaries are defined by an envelope constituted by the cell wall and the plasma membrane. The cell wall, which is in the outermost layer of this envelope, is formed by a net of polysaccharides and proteins, and it is freely permeable to small molecules like water. On the other hand, the plasma membrane is composed by an apolar bilayer of lipids linked only by hydrophobic interactions, and inserted proteins, representing a diffusional barrier for water and being relatively impermeable for large or hydrophilic/charged molecules. As a consequence, a packed mass of yeast has a percentage of extracellular water, which surrounds the cells, and another of intracellular water, enclosed inside them [1].

Direct observation in microscopy [2], methods using radioactive labelled substances like $^{3}H_2O$ and [^{14}C]sorbitol [3], or $^{3}H_2O$ and *L*-[U-^{14}C]glucose [4]; the dye method [5] and the density method [6], are different techniques employed for the evaluation of the intracellular water content, which is a determinant factor of the

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physiological properties of living cells. Another method, proposed by Uribelarrea [7], consists in a thermogravimetric analysis of the drying process of packed microorganisms, using an infrared balance at 80°C.

Particulated solid drying usually occurs in three stages [8, 9], as shown in Fig. 1, which shows a typical qualitative plot of the drying rate as a function of the residual water content. A first increasing drying rate step occurs until the desired drying temperature is attained, which ends at point A. From this point on, there is a constant drying rate step until the total loss of free water at point B, after which all the particles are in direct contact with each other. After this condition, the drying process begins to depend on the diffusion of the water from the internal pores between particles (interstitial water) to the surface of the material, causing a continuous falling rate period as the water content decreases.

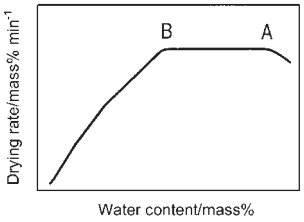

Fig. 1 Typical qualitative drying curve of particulated solid materials

The analysis of the isothermal drying curve of wet packed cells, through a plot of the drying rate *vs*. the residual mass as suggested by Uribelarrea [7], was used in this study for the determination of intracellular water content of microorganisms by using isothermal termogravimetric analysis in conventional equipment. The differences on the characteristics of the resulting drying curves when compared to the one shown in Fig. 1, are discussed with respect to the cell drying process. Much smaller samples than in infrared thermogravimetry may be used and the results are coherent with those measured by infrared and other conventional techniques.

Materials and methods

The analyzed microorganism was the yeast *Saccharomyces cerevisiae* (commercial yeast – Fleischmann). A certain amount of biomass was suspended in distilled water and centrifuged at 3000 rpm for 10 min. This procedure was repeated 3 times with the purpose of eliminating soluble impurities in the supernatant.

Samples from about 7 to 18 mg of washed biomass were analysed in a TA-Instruments simultaneous TG-DTA, model SDT-2960 equipment which allows mass measurements with a precision of 0.001 mg. Aluminium pans and 60 $cm^3 s^{-1}$ air flow rate were used. Starting from room temperature with a heating rate of 10°C min^{-1} the temperature was maintained at 60 or at 80°C. When the mass loss rate was practically zero, the temperature was raised to 125°C for about 7 min in order to complete the water elimination and to determine the water free residual mass (dry

mass – M_d). Considering M_o the initial mass, and M the residual mass at a time t, a dM/dt *vs.* M curve was plotted, based on the conventional thermogravimetric analysis data obtained for each sample. The mass concerning to the point where a typical change in the curve behavior occurs, as observed in infrared thermogravimetry [7], was defined as the mass of the cell free of extracellular water (M').

The measured values were used to calculate the intracellular water content (P_{iw}), and the dry mass content (P_d). These contents are calculated on an extracellular water free M' basis, which represents the sum of the respective masses M_{iw} and M_d as shown below:

$$M_{iw}=M'-M_d \text{ intracellular water mass}$$

$$P_{iw}=100\frac{M_{iw}}{M'} \quad \text{percentual intracellular water content}$$

$$P_d=100\frac{M_d}{M'} \quad \text{percentual dry mass content}$$

Additionally, we can calculate the value of the extracellular water mass (M_{ew}) and extracellular water content (P_{ew}) using the same base M', which allows the comparison between these values :

$$M_{ew}=M_o-M' \text{ extracellular water mass}$$

$$P_{ew}=100\frac{M_{ew}}{M'} \quad \text{percentual extracellular water content}$$

Results and discussion

Figure 2 shows a typical mass loss curve obtained during an isothermal thermogravimetric analysis performed at 60°C and the corresponding derivative curve as a function of time (case 1). It can be seen that in the first five minutes the weight loss rate, which represents the drying rate, has increased significantly because of the initial transient temperature change from ambient temperature to 60°C, during which, the heating rate is increased automatically by the equipment. A decreasing drying rate follows this step instead of the constant drying

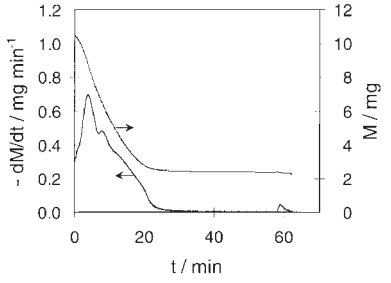

Fig. 2 Typical thermogravimetric curves of a yeast sample at 60°C

rate usually seen for particulated wet materials presenting free water. This is also shown for the same sample in Fig. 3, where the drying rate is plotted *vs*. the residual mass as suggested by Uribelarrea *et al*. [7], to identify and correlate the different type of cellular water loss during the drying process with the changing cell mass.

It is very important to note that the samples were centrifuged before the analysis. This procedure corresponds to a first significant drying step of the cells, after which all the cells are in contact to each other, having only some residual extracellular interstitial water between them. This may explain why right after the initial transient stage it is seen a decreasing drying rate step, as it occurs during particulated material drying when no more free water is present.

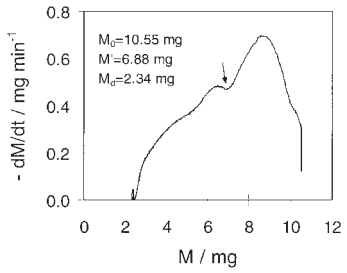

Fig. 3 Case 1 – Drying curve of a yeast sample at 60°C

As indicated with an arrow in Figs 2 and 3, there is a critical point in the drying curve when there is no more interstitial extracellular water [7], and the intracellular water begins to be released at a drying rate which slightly increases for a brief period, after which it again decreases significantly until complete dryness of the cell. Our interpretation to this fact is that at the critical point, the porosity of the external surface of the cells is somehow changed, increasing its permeability, which enables, in a second transient period, the loss of the intracellular water more easily than in the subsequent drying process.

Figure 4 shows an analysis at 80°C of another sample (case 2) with a similar drying curve behaviour. The time needed to achieve a drying rate near zero was of about 5 min in this case. Figure 5 shows an experiment done at 60°C, where an additional amount of water was added to a centrifuged sample before the analysis

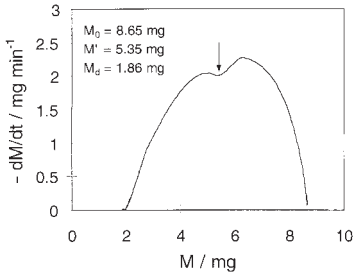

Fig. 4 Case 2 – Drying curve of a yeast sample at 80°C

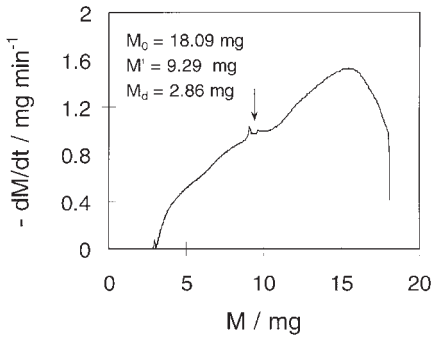

Fig. 5 Case 3 – Drying curve of a yeast sample with added extracellular water at 60°C

(case 3). As can be seen, also in this case there is no practically a constant drying rate period after the maximum mass loss rate is attained, indicating apparently that this complementary added water was not sufficient to have a 'free' water. This procedure has not altered significantly the respective P_{iw} and P_d values, but as expected, a greater P_{ew} value is obtained as shown in Table 1, where also other case data are shown. The results also show that P_{iw} and P_d are consistent with each other and that they do not depend on the initial mass of the samples.

Table 1 Measured and calculated data obtained from drying curves of yeast samples

Case	Operating temperature/°C	M_{iw}/mg	M_{ew}/mg	P_d/mass%	P_{iw}/mass%	P_{ew}/mass%
1	60	4.56	3.67	34	66	53
2	80	3.49	3.30	35	65	62
3	60	6.44	8.80	31	69	95
4	80	7.24	6.39	33	67	59
5	60	2.50	2.39	34	66	63
		Mean values		33	67	–

In the Uribelarrea *et al*. [7] method with infrared balance, samples of about 1 g were used and in this work much smaller available sample mass may be analyzed. The use of a conventional TG equipment with air flow, allows a shorter analysis time (about 20 min for a 80°C analysis), because the partial pressure of the released water vapor is maintained at low levels. One drawback of this method is that the cells are killed in the process, what may hinder its further use in studies where it is needed to maintain them alive after or on the course of the experiment. However, considering that it is used a very small sample, thermogravimetry with conventional equipment using the method developed by Uribelarrea is also a good option to the determination of intracellular water content of microorganisms.

Conclusions

Conventional thermogravimetric equipment can be used for the determination of intracellular water content of yeast samples, using much smaller sample masses and operating times than previous developed methods.

The values obtained for *Saccharomyces cerevisiae* samples by conventional thermogravimetry with air flow, are coherent with those measured by other techniques, and do not depend on the initial sample mass and operating temperatures, within the respective experimental ranges used in this work.

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