Acta Biologica Szeged. *23* (1—4), pp. 83—87 (1977)

COMPARISON OF METHODS FOR DETECTION OXYGEN UPTAKE IN YEASTS: THE AUTOMATIC COULOMETER AND THE WARBURG APPARATUS

I. GAÁL and J. SOÓS

Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, Szeged Department of Comparative Physiology, Attila József University, Szeged

(Received October 20, 1976)

The oxygen uptake of four different yeast species has been determined by using an automatic coulometer as well as Warburg's method. While the two methods give nearly the same rate of oxygen consumption, small changes can be followed only when coulometric measurements are employed. The resulting oxygen uptake curves were fitted with a polynom of third degree. Automatic coulometer has been found to provide not only extremely accurate but also convenient tool in determining oxygen uptake. The method seems to be generally applicable to the study of gas uptake of different biological and chemical systems.

Introduction

Oxygen uptake of biological systems is determined generally by manometric methods, in most cases using Warburg apparatus. When studying certain fine processes, particularly when the oxygen consumption in a reaction is to be unrevealed, however, the Warburg method is not sensitive enough.

In order to overcome the experimental difficulties DOBOS and GAÁL (1974) constructed an automatic coulometer which conveniently and precisely measures the uptake of small amount of gases. It is an especially useful tool when one is interested in the mechanism of symultaneous oxygen and hydrogen uptake. The consumed gases are supplied by electrolysis therefore the amount of reacted gases can be estimated from the coulombs needed to maintain the equilibrium states. As the measurement is reducted to a simple measurement of time, the method is easy to automatize.

In the present paper a comparison of the utility of the two method is given. The applicability of the Warburg as well as the automatic coulometer methods are established in yeast model system.

Materials and Methods

The following yeast strains have been used: Saccharomices cerevisiae RXII., Candida utilis, Candida utilis major, Saccharomices cerevisiae EB4.

The yeasts have been cultured for 24 hours at 25 °C with continuous shaking. The culturing medium contained 5 g oxoide (Difco) and 10 g glucose dissolved in 1000 ml water.

The dry weights have been determined on an analytical balance after keeping the product at 110 °C in a desiccator filled with P_2O_5 for 2 hours.

Detection of mitochondria:

Nitro-BT dye has been used which reacts with the succinate-dehydrogenase in mitochondria. The product forms a blue colored complex. The cells have been incubated at 37 °C for 15 minutes in the following medium:

Sodium succinate	0,06 molar	1,0 ml
Nitro-BT dye	0,2 molar	2,5 ml
Phosphate buffer	0,2 molar	1,0 ml
KCl	0,6 molar	0,5 ml

Results and Discussion

The generally used technics have been applied to the determination of oxygen uptake in the Warburg apparatus. The wet weight of our initial sample was 0.01 g. Temperature was kept at 30 °C. Coulometric measurements were carried out under the same circumstances. Table 1. summarizes the oxygen uptake of the four yeast strains.

It is apperent from Table 1 that Sacch. cer. EB4 does not consume oxygen at all according to the coulometric method. The value obtained in the Warburg apparatus lies within the experimental error.

Table 1. Oxygen uptake of the studied yeast strains as measured in a Warburg apparatus and in a coulometer, respectively.

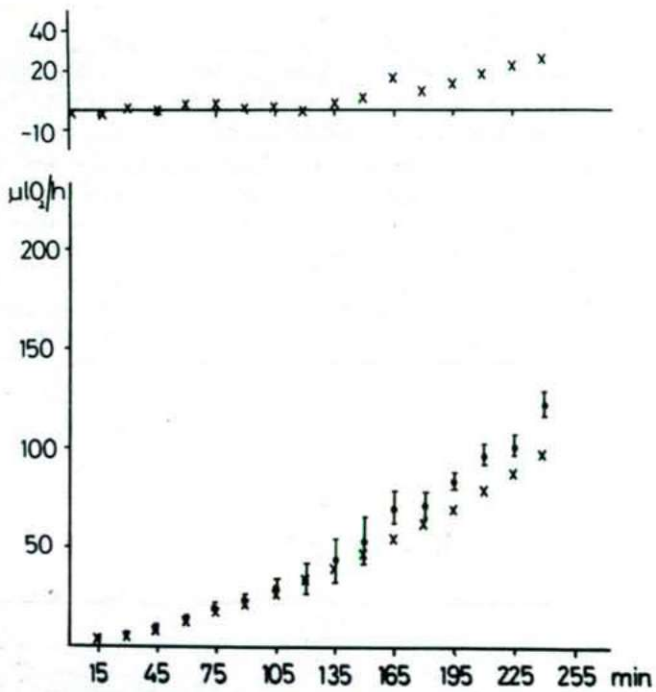

Fig. 1. Oxygen uptake of the Sacch. cer. RXII. strain in time obtained on the Warburg apparatus.

Table 1

Strain	Oxygen uptake microliter/mg dry weight hour	
	Warburg apparatus	Coulometer
Sacch. cer. RXII	101	135
Candida utilis major	85	97
Candida utilis	221	205
Sacch. cer. EB4	1,97	0,00

Sacch. cer. RXII has been selected for a comparison of the two methods in a time dependent oxygen uptake study.

Figure 1 shows the results collected on the Warburg apparatus. The experimental points are labeled with rings while the points of the corresponding third degres polynomial firt are denoted with x. The deviation of the experimental points is indicated by vertical rods. Each point represents an average of three measurements. It can be seen that the third degree polynomial fit describes the experimental pattern very well until the reaction time is short enough.

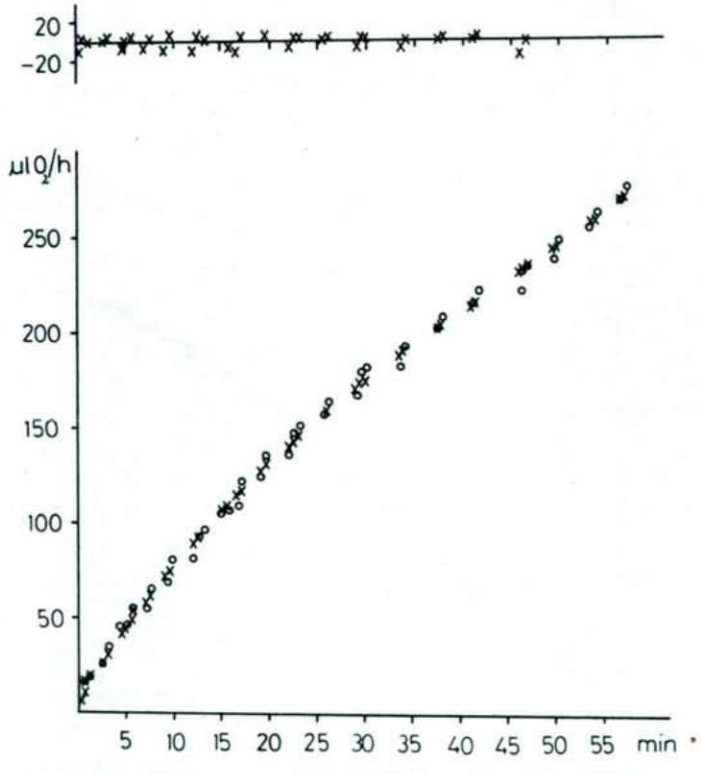

Fig. 2. The same as measured with the coulometric method.

The deviation of the two curves becomes pronounced only after a longer reaction time. The parameters of the polynom were computed using the least square method. Values in Figure 1 are not corrected for the cell number growth in time, therefore they differ from those given in Table 1.

The third degree polynom is given as where b_0, b_1, b_2, and b_3, are the respective regression coefficients.

In Figure 2 the oxygen uptake of the same yeast culture is plotted as measured by the coulometric method. Denotations are the same as in Figure 1.

A comparison of the two figures reveals that the coulometric method is more accurate and as a result the points are better fitted with the third degree polynom. In contrast to the curve obtained with the Warburg apparatus, here the computed values does not differ from the experimental ones even at long reaction times.

We have found in previous studies that ethidium bromide inhibits oxygen uptake. The mechanism of this phenomenon was investigated with the more exact coulometric method.

Administering ethidium bromide at the start of the experiment will produce a stable state by the 15th minute which remains unchanged during the next 24 hours. The observation is in line with the hypothesis that the principal effect of ethidium

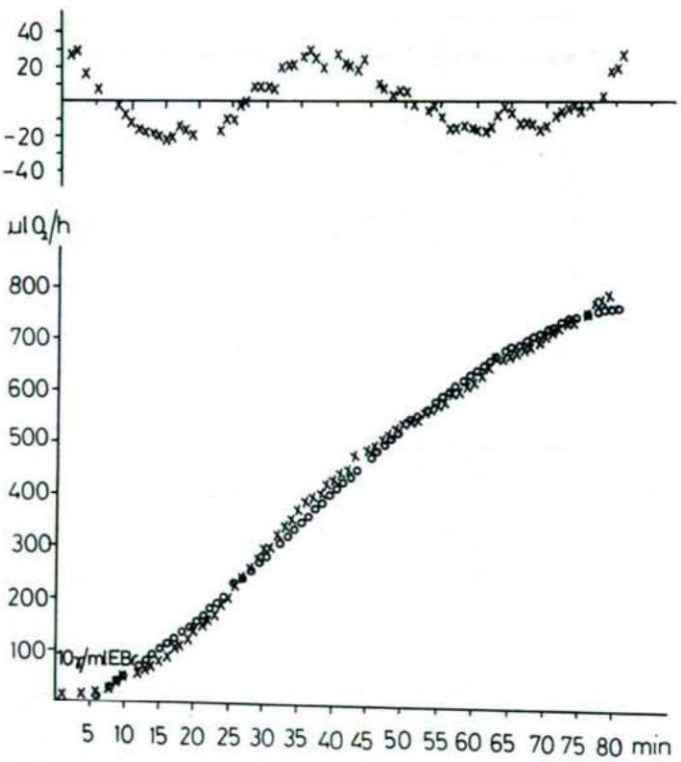

Fig. 3. Coulometric study on ethidium bromide inhibition of oxygen uptake in time. EBr concen t ration 10^{-5} g/ml.

bromide is degradation of mitochondrial DNA. Since several important enzymes of respiration are partly or wholly coded in mitochondrial DNA ethidium bromide does affect respiration but only following a delay phase. This is the time needed for the decomposition of the enzymes as well as the DNA. In two days respiration deficient mutants can be isolated displaying no respiratory activity.

One can see in Figure 3 that adding ethidium bromide in the 5 th minute of the experiment decreases reaction rate during the next 15 minutes then oxygen uptake returns to close to normal. Such small change is not detectable in the Warburg apparatus.

Acknowledgement. The authors wish to thank Miss ANNA MARÁZ of the Institute of Microbiology of JATE for allowing to use the Warburg apparatus as well as for her kind help.

References

DOBOS L.—GAÁL I. (1974): Versatile Automatic Coulometer. – Acta Biologica Szeged. *20*, 129—135.

Address of the authors:
Dr. I. GAÁL
Department of Comparative Physiology,
A. J. University,
H—6701 Szeged, P. O. Box 428,

Dr. J. Soós
Institute of Biophysics,
Biological Research Center,
Hungarian Academy of Sciences,
H—6701 Szeged, P. O. Box 521,
Hungary