Acta Bot. Neerl. 21(2), April 1972, p.149–154.

DETERMINATION OF THE YEAST CELL pH

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SUMMARY

Once freezing and thawing the yeast leads to incomplete destruction of the cell membrane and to too low values of the cell pH determined in the cell suspension with the glass electrode. The cells are rendered completely permeable through boiling, treatment with acetone, or freezing and thawing the cell repeatedly. Cell pH's obtained in this ways are definitely higher. The differences in cell pH's observed with completely broken cells and partly broken cells can be accounted for quantitatively to the presence of intact cells in the only once frozen and thawed cell suspension.

1. INTRODUCTION

Generally yeast cell pH values are obtained with a glass electrode in the cell suspension after freezing and thawing the cells once (CONWAY & DOWNEY 1950; SUOMALAINEN & OURA 1955 and IDZIAK & WIKEN 1962). This procedure leads to lower pH values than when the cells are boiled before determining the pH (CONWAY, BRADY & CARTON 1950; BRANDT 1945). We will now give evidence that these differences are due to incomplete destruction of the cells by the freezing-thawing method. A large part of the cells still remains intact after applying this procedure.

2. METHODS

2% w/v yeast *Saccharomyces cerevisiae* Delft II is preincubated for 60 minutes in 0.1 M sodium citrate buffer pH 4.5 provided with 10 mM KCl in the presence of 3% glucose under anaerobic conditions. Four samples of 10 ml of yeast suspension are filtered by suction through Hirsch funnels provided with a Schleicher and Schüll No 602h filterpaper of 2.7 cm diameter, and the cells are washed with 3 ml of icecold water in order to remove the adhering buffer. These cells are either washed on the filter with 3 ml of acetone in order to obtain acetone powders or the filters with the cells are put into liquid nitrogen for at least 6 minutes. Cell pH's are determined with a combination glass electrode in a suspension consisting of four filterpapers with the yeast cells on them and either 1.5 ml of water when it concerns the acetone powders or 0.5 ml of water when frozen cells are thawed. These small amounts of water have to be added in order to make it possible to carry out accurate determinations with the glass electrode. The pH's are determined within 30 seconds When the frozen or acetone treated cells are boiled before determining the pH these cells are heated

for 30 seconds just to 100°, and are then cooled rapidly to room temperature, whereafter the pH is determined immediately

The amount of water of the cells which is inaccessible to mannitol is determined by adding 2.0 ml of 1 mM ^{14}C labelled mannitol from Philips Duphar to the cells from one filter paper, after removing them from the filterpaper. The cells are centrifuged after 2 minutes and after separating the supernatant the residue is extracted for 4 minutes at 60° with 2 ml of 0.2 M bicarbonate. 0.5 ml of both the bicarbonate extract and the supernatant are assayed for radioactivity by means of liquid scintillation (BORST-PAUWELS 1968). The amount of water which is inaccessible to mannitol is equal to the difference of the number of grams of water present in the residue and the amount of water in the residue into which mannitol has penetrated. The latter amount equals the quotient of the total radioactivity of the residue and the radioactivity of 1 gram of the supernatant.

Staining of the treated cells with bromophenolblue is performed by incubating the cells in a solution of 0.1 mM bromophenolblue in the citrate buffer of pH 4.5 for a few minutes. The number of stained cells are determined with a hemocytometer.

3. RESULTS

The amount of cell water which is not accessible to mannitol varies with the treatment of the cells. This figure decreases to about 70% of the value observed with whole cells after freezing and thawing the cells once and to approximately 3–20% after boiling the cells or after washing the cells with acetone. Repeatedly freezing and thawing the cells renders them also quite permeable to mannitol. Whole cells appeared to be completely impermeable to bromophenolblue. Freezing and thawing the cells once makes about one quarter of the cells permeable to this dye. Repeatedly freezing and thawing the cells, boiling them, or washing them with acetone renders almost all cells permeable to bromophenolblue. *Table 1* shows that a lower pH is observed with suspensions of cells which are only partly permeable to bromophenolblue or to mannitol than with suspensions the greater part of which is made permeable to these solutes.

As shown by the data of *table 2*, mixing intact cells with completely broken cells in about the same proportion as is found in cell preparations once frozen and thawed leads to a pH value not differing much from the value observed after freezing and thawing once. The small difference between the two pH's can almost completely be accounted for by the fact that in reality the percentage of cells which are still intact equals 79% instead of 75%. When the difference between the pH observed with whole cells and with partly broken cells is linearly related to the percentage of cells which are completely broken, a pH of 6.521 is calculated for the case that only 21% of the cells are made permeable to small solutes.

The incomplete breaking of the cells membranes which apparently occurs after freezing and thawing once is not due to too short a period of freezing.

Table 1. Effect of treatment upon measured pH, mannitol inaccessible space and upon the number of cells stained by bromophenolblue.

Treatment	pH	SE	n	MIW	SE	n	%	SE	n
				ml gr^{-1}					
no				1.691	0.043	3	0	0	4
1 × fr.th.	6.58	0.035	7	1.196	0.152	3	21.3	1.2	3
10 × fr.th.	7.11	0.010	3	0.321	0.118	3	92.8	1.4	4
1 × fr.th., boiled	7.03	0.031	10	0.152	0.025	3	97.3	1.0	4
acetone	7.31	0.064	9	0.399	0.010	3	100	0	4
acetone, boiled	7.16	0.043	9	0.049	0.060	3	100	0	4

MIW: mannitol inaccessible water in ml per gram of dry weight of acetone extracted yeast.
SE: standard error
n: number of determinations.
%: number of cells which are stained by bromophenolblue.
no: the cells are washed only with water; fr.th.: the cells are frozen and thawed; acetone: cells are washed with acetone; boiled: the cells are boiled for 30 seconds after the appropriate treatment.

Table 2. Comparison of pH values obtained after freezing and thawing the cells once and after mixing untreated cells with boiled cells.

untreated cells %	100	75	0	0
1 × fr.th., boiled cells %	0	25	100	0
1 × fr.th., cells %	0	0	0	100
pH	6.423	6.540	6.853	6.505
SE	0.013	0.016	0.010	0.006
n	4	4	4	4

see also legend to table 1.

It is observed that an increase of the period of freezing in liquid nitrogen from 6 minutes up to 24 hours does not increase the measured cell pH.

We have also examined whether the differences between cell pH observed after freezing and thawing once and the value obtained after boiling the cells depends upon the state of the cells. It is seen from *fig. 1* that this difference is smaller with resting cells than with metabolizing cells. The percentage of cells which are stained by bromophenolblue does not differ significantly from that found with metabolizing cells. Addition of glucose leads to an almost immediate increase in cell pH with metabolizing cells, whereas this pH increases with a lag time or decreases even for a short period according to the method of freezing and thawing once. Addition of 2,4-dinitrophenol to metabolizing cells in a concentration at which several energy requiring processes are stopped (RIEMERSMA 1968; JARETT & HENDLER 1967; BORST-PAUWELS & JAGER 1969), whereas glycolysis is virtually unaffected, leads to an immediate decrease in cell pH. With the freezing and thawing method a partial recovery of the cell pH is found which is not observed when the cells are boiled before measuring the pH.

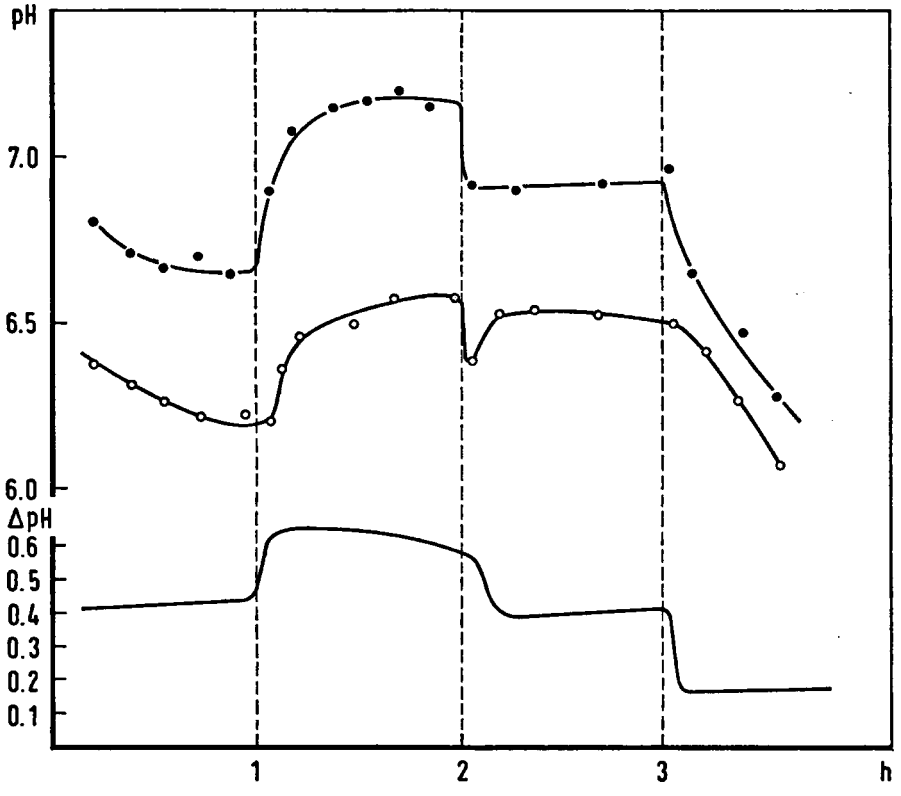

Fig. 1. Effect of varying metabolic conditions upon the yeast cell pH as determined according to the freezing and thawing method (○) and after washing the cells with acetone followed by boiling (●). The lower curve represents the difference between the two pH's. After one hour incubation in the absence of glucose, 3% glucose was added to the yeast suspension. One hour later 0.1 mM 2,4-dinitrophenol is added and at 3 hours 1 mM iodoacetate is added.

As a consequence the differences in pH values obtained with both methods decrease with time. Iodoacetate which stops glycolysis gives rise to a further decrease in cell pH. The differences between pH values observed with both methods decrease still further.

4. DISCUSSION

One of the main arguments of CONWAY & DOWNEY (1950) that the pH measured after freezing and thawing the cells once is the true one, is that the partition of acetic acid between resting cells and medium is just of the order of magnitude which is expected for that pH and is much too small if the pH obtained after boiling the cells were the true one. This argument is based upon the assumptions that the acetic acid passes the cell membrane only in its undissociated form and

that all compartments of the cell are accessible to the acid. When one of these two assumptions is not true their argument is no longer valid. As a matter of fact one should be very careful to use figures concerning acid distribution between cells and medium in order to prove that the pH determined by a direct method via the glass electrode is the true one. This is only permitted when one is dealing with cells which are not compartimentalized. We have now shown that freezing and thawing the cells once is an insufficient means for breaking the membranes of most cells. About 80% of the cells is not stained by bromophenolblue after this treatment and the amount of cell water which is not accessible to mannitol is still very high. Boiling the cells does not affect the value of the cell pH, as is shown by the fact that the same pH value is found after repeatedly freezing and thawing the cells, a method by which all cells are made almost completely permeable to mannitol and bromophenolblue as well.

The single freezing and thawing method does not only give rise to too low estimates of the cell pH, but also the time course of the changes in cell pH with changes in the metabolic state of the cell might be different from those observed after completely breaking the cell membranes, as shown in *fig. 1.*

CONWAY, BRADY & CARTON (1950) also found that the difference between the pH values observed after boiling the cells and after freezing and thawing them once is larger with metabolizing cells than with resting cells. This might be due to the fact that the intact cells which are still present in the suspension lead to a greater acidification when it concerns metabolizing cells than when one is dealing with resting cells. This provided us also with an explanation for the effect of 2,4-dinitrophenol upon the difference between the two pH's. This uncoupler stops the proton pump (RIEMERSMA 1968). Iodoacetate has a similar effect and reduces the difference between the two pH's as well.

One might question whether it is necessary to freeze the cells before boiling them. As a matter of fact BRANDT (1945) boiled the cells immediately without freezing them previously. It is much more practical, however, to freeze the cells first, because one can keep the cells for a long period in the frozen state, thus making it possible to take a large number of samples within a relatively short time.

ACKNOWLEDGEMENT

The authors are much indebted to the Gist- en Spiritusfabriek, Delft for providing the yeast.

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