MECHANISM OF SUCROSE UTILISATION BY *SACCHAROMYCES CEREVISIAE*

PATRICK K. MWESIGYE* AND JOHN P. BARFORD

Department of Chemical Engineering, University of Sydney, NSW 2006, Australia

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Saccharomyces cerevisiae was grown on different concentrations of sucrose and glucose mixtures after adapting on sucrose. The yeast cells were found to have two different mechanisms by which sucrose was utilised: hydrolysis outside the cell membrane and direct transport into the cells. The mechanism by which sucrose was utilised depended on the initial concentration of glucose in the mixture and the adaptation state of the cells. In both cases, glucose was utilised first and invertase secretion was repressed when the glucose concentration was higher than 2 g l^{-1} . The major important finding in our results is that, for fully sucrose-adapted cells, even in the presence of a repressive glucose concentration, the yeast cells were able to utilise sucrose, and most importantly, without first being hydrolysed to its constituent monosaccharides. Previously, it has been thought that direct transport of sucrose contributes only a small part of the process of utilisation of sucrose, but as our results indicate it can be the major mechanism of sucrose utilisation in *S. cerevisiae* if the cells are adapted on sucrose for long periods, glucose concentrations in the medium are high and/or invertase activities are limiting.

The initial steps in the utilisation of sugars by yeast is either the passage of intact sugar molecules across the cell membrane or the hydrolysis outside the cell followed by entry of all or some of the hydrolytic products into the cell. Sugar transport into microbial cells across the plasmalemma can take place by active transport, facilitated diffusion and group translocation (19).

Sucrose utilisation involves its hydrolysis outside the yeast cell by extracellular enzyme invertase (β -D-fructosidase) into glucose and fructose which are then transported into the cells and metabolised. Most *Saccharomyces cerevisiae* strains have two forms of invertase: an external large invertase, which is a glycoprotein, and an internal small invertase (9,15). Some strains of *S. cerevisiae* have been reported to transport sucrose directly into the cell (14,17,20).

* Address reprint requests to: Dr. Patrick K. Mwesigye, Department of Chemical Engineering, University of Sydney, NSW 2006, Australia.

In a number of countries (e.g. Australia, Mauritius and South Africa), sucrose is employed as a wort adjunct. The presence of sucrose significantly modifies the initial wort sugar spectrum and also the pattern of wort sugar uptake during fermentation (5, 7). The effect of glucose (catabolite) repression on the uptake of sugars in yeasts is well reported (for review, see ref. 8). The mechanism by which sucrose is utilised by *S. cerevisiae*, especially in the presence of other sugars is not well understood and a reappraisal of the sucrose transport mechanism is still required. In this paper, we have studied the regulation of sucrose utilisation and invertase in the presence of glucose. A new approach to the mechanism for utilisation of sucrose is also proposed.

MATERIALS AND METHODS

Organisms. The strain of baker's yeast used in this work was *Saccharomyces cerevisiae* 248 UNSW 703100 (3, 14, 17). This yeast was maintained on malt agar slopes and stored at 4°C.

Growth medium. Fully defined growth medium with the following composition ($g\ l^{-1}$): $(NH_4)_2SO_4$, 5.00; KH_2PO_4 , 3.40; Na_2HPO_4 , 0.41; citric acid, 0.21; $MgCl_2 \cdot 6H_2O$, 1.4×10^{-2} ; $CaCl_2$, 3.5×10^{-3} ; $MnCl_2 \cdot 4H_2O$, 2.5×10^{-3} ; $ZnCl_2$, 2.0×10^{-3} ; $CoCl_2$, 6.0×10^{-4} ; $CuCl_2 \cdot 2H_2O$, 4.0×10^{-4} ; H_3BO_3 , 2.0×10^{-4} ; biotin, 8.0×10^{-4} ; *p*-amino benzoic acid, 8.0×10^{-3} ; riboflavin-5-phosphate, 8.0×10^{-3} ; calcium pantothenate, 1.6×10^{-2} ; nicotinic acid (niacin), 1.6×10^{-2} ; pyridoxine hydrochloride, 1.6×10^{-2} ; thiamine, 1.6×10^{-2} ; inositol, 8.0×10^{-2} . The composition is based on $10\ g\ l^{-1}$ of total sugar concentration and was proportionally increased when the initial total sugar content was increased. The sugar solution, consisting of different proportions of sucrose and glucose (in case of mixtures), was autoclaved separately and the filter-sterilised mineral and vitamin solutions added aseptically. All chemicals were of analytical grade.

Adaptation of yeast cells. Adaptation of yeast cells on sucrose was carried out by transferring the cells from agar slopes into 250-ml shake flasks containing about 100 ml of liquid growth medium. The flasks were then incubated in a Gallenkamp orbital incubator (model IH460) at 250 rpm and 30°C. Transfers of between 0.1 and 1.0 ml were made at approximately 12-h intervals to ensure that the cell viability remained above 99%. This process of adaptation of the yeast cells to sugar in the medium was carried out for 2 to 14 days (about 27 to 250 generations). An actively growing inoculum, taken during the exponential growth phase, was used in all experiments and was about 0.05% (w/v) of the total growth medium in the fermenter.

Growth conditions. The growth conditions in a batch fermenter with a 4-l working volume were strictly defined. The oxygen was maintained at greater than 75% saturation, temperature at $30 \pm 1^\circ C$ and pH at 5.0 ± 0.1 . The temperature was maintained using either cooling water or infrared heating lamps (250 W, Philips, Sydney, Australia) and the pH by automatic addition of 2 M sodium hydroxide

solution. These parameters were controlled on-line and the data logged using an FC-4 control system (Real Time Engineering, Sydney, Australia).

Dry weight determination. At specified times during growth, 20 ml of yeast cell suspension was withdrawn from the fermenter. The samples were filtered through preweighed membrane filter papers ($0.45\ \mu\text{m}$, Gelman Sciences, Ann Arbor, MI, U.S.A.) and the filtrate collected for sugar and ethanol analysis. The filter papers were dried in an oven at 105°C for 30 min. They were then weighed after cooling in a desiccator.

Sugar and ethanol analysis. High-performance liquid chromatography (HPLC) was used to analyse sugars and ethanol in the fermentation samples using a Waters Shodex SC 1011 column. The HPLC set up consisted of Waters pump model 510, WISP model 710A autosampler and refractive index detector (model R401). The mobile phase was deionised water purified using Barnstead NANOpure II purifier and degassed by sonication. It was pumped at a constant flow rate of $0.8\ \text{ml min}^{-1}$. The column was maintained at 85°C . Data was acquired, stored and chromatograms integrated using Delta Junior software (Digital Solutions, Brisbane, Australia). The samples for HPLC analysis were prepared using Sep-Pak C18 Plus cartridges (Waters, Milford, U.S.A.).

Gas analysis. The percentages of oxygen and carbon dioxide in the exit gas from fermenter were measured using Taylor Servomex analyser (type O.A. 137) and M.S.A Lira infrared analyser (model 303), respectively. To eliminate inaccuracies due to the presence of water vapour, the exit gas was chilled to $1-3^\circ\text{C}$ using a Hartmann and Braun gas cooler (model CGEK) prior to analysis.

Calculation of metabolic parameters. The major metabolic parameters included specific growth rate (μ), biomass yield (Y_{xs}), ethanol yield (Y_{e}), specific sugar uptake rate (Q_{s}) and specific ethanol production rate (Q_{e}) and were calculated as previously described (16).

Specific growth rate, μ , was obtained from the slope of semi-logarithmic plot of the dry weight (X) versus time and the maximum specific growth rate in batch obtained by linear regression. The overall biomass yield, defined as the amount of biomass produced per gram of sugar utilised, was approximated from:

$$Y_{\text{xs}} = \frac{\Delta X}{\Delta S} = \frac{X_2 - X_1}{S_1 - S_2}$$

where X_1 , X_2 are dry weights and S_1 , S_2 are the corresponding residual sugars in the fermenter.

Ethanol yield, Y_{e} , the amount of ethanol produced per gram of biomass of yeast cells was approximated from:

$$Y_{\text{e}} = \frac{\Delta E}{\Delta X} = \frac{E_2 - E_1}{X_2 - X_1}$$

Y_{e} = ethanol yield ($\text{mmol g-biomass}^{-1}$)

E_1 , E_2 are ethanol concentrations (mM) and X_1 , X_2 are the corresponding biomass concentrations ($g\ l^{-1}$) in the fermenter.

Overall yields during exponential growth were calculated rather than incremental yields as the accuracy of sugar and ethanol analyses was not high enough for consistent calculation of incremental yields.

The specific sugar uptake rate was calculated from:

$$Q_s = \frac{\mu}{Y_{xs}}$$

Q_s = specific sugar uptake rate ($g\text{-sugar}\ g\text{-biomass}^{-1}\ h^{-1}$).

Hence, Q_s can be obtained from the specific growth rate and the biomass yields. For purposes of comparing the specific sugar uptake rates when the cells were growing on different sugars, Q_s was mainly expressed in terms of equivalent mmoles of glucose $g\text{-biomass}^{-1}\ h^{-1}$. Similarly, the specific ethanol production rate was calculated from:

$$Q_e = \mu Y_e$$

Q_e = specific ethanol production rate ($mmol\ g\text{-biomass}^{-1}\ h^{-1}$).

Invertase assay. Cell suspensions were withdrawn from the fermenter at specified time intervals. The cells were harvested and washed twice with distilled water by centrifugation at $3,500 \times g$ for 15 min. Harvested cells were resuspended in distilled water and then used directly for external invertase assay. Invertase in yeast cells was assayed as previously described (9). The invertase assay was carried out by adding 50 μ l of yeast cell suspension to 100 μ l of 0.1 M sodium acetate buffer pH 5.0 and 50 μ l of sucrose solution ($100\ mg\ l^{-1}$). The final yeast cell density in the incubation mixture was $1\ mg\ dry\ weight\ yeast\ cells\ m^{-1}$. The mixture in test tubes was incubated for 10 min in a water bath at $30^\circ C$. The reaction was stopped by the addition of 200 μ l of 0.1 M dibasic potassium phosphate (K_2HPO_4) buffer pH 7.0 and the tubes were immediately placed in a boiling water bath for 3 min. The tubes were cooled and glucose liberated was assayed.

Glucose liberated from sucrose hydrolysis was assayed using glucose hexokinase kit (Hoffman-Roche, Basel, Switzerland). One hundred microliters of the incubation mixture was added to 900 μ l of the hexokinase enzyme and incubated for 10 min after thorough mixing. Absorbance was read at 340 nm (Unicam SP 1800 UV spectrophotometer) within 30 min. For each of the samples assayed for invertase, sucrose and enzyme blanks and glucose standards were included.

One unit of invertase (U) is the amount (μ mol) of glucose released when sucrose is hydrolysed at $30^\circ C$ in 1 min in 0.1 M sodium acetate buffer pH 5.0. Specific invertase activity is expressed as U mg-dry wt $^{-1}$.

RESULTS AND DISCUSSION

Regulation of sucrose utilisation

The yeast cells were adapted on sucrose for 2 or 14 days and then grown on a mixture of sucrose and glucose in a batch fermenter. The results of sugar profile utilisation and growth on the mixture with different proportions of glucose and sucrose after adaptation on sucrose for 2 and 14 days are shown in Figs. 1 and 2. The profile of sugar utilisation shown in Fig. 1 was characteristic of growth of cells when the glucose concentration in the mixture was 10 g l^{-1} or less. It should be noted that the sugar concentrations shown in Figs. 1 and 2 are those present at the time of sampling (after the cells have attained exponential growth) and are necessarily less than the initial sugar concentration at the time of inoculation. The lag to early exponential growth phases after inoculum was about 6 to 8 h long and during this period, some of the sugars in the mixture were utilised. Secretion of

Fig. 1. Sugar utilisation profile and growth of *Saccharomyces cerevisiae* 248 UNSW 703100 on mixture of 10 g l^{-1} sucrose and 10 g l^{-1} glucose after adaptation on sucrose for (a) 2 days, (b) 14 days.

This sugar utilisation profile was characteristic of sugar mixtures containing 1% (w/v) glucose or less. Symbols: sucrose ($\circ$), glucose ($\bullet$), fructose (∇), ethanol ($\blacktriangledown$), dry weight ($\blacksquare$).

Fig. 2. Sugar utilisation profile and growth of *Saccharomyces cerevisiae* 248 UNSW 703100 on a mixture of 8 g l^{-1} sucrose and 12 g l^{-1} glucose after adaptation on sucrose for (a) 2 days, (b) 14 days.

Symbols: sucrose (○), glucose (●), fructose (▽), ethanol (▼), dry weight (■).

invertase resumed after the glucose concentration fell below 2 g l^{-1} and sucrose was hydrolysed to glucose and fructose. However, when the cells were growing on mixture containing more than 10 g l^{-1} glucose at inoculation, sucrose was utilised without first being hydrolysed to its constituent monosaccharides (Fig. 2). This is confirmed by the absence of fructose in the growth medium. For cells adapted for 2 days, sucrose was utilised after almost all the glucose was exhausted (Fig. 2a). For the fully adapted cells, sucrose was consumed simultaneously with glucose but at a lower rate. This could mean that there is a sucrose transport system that is induced when the cells are adapted on sucrose for a long time (up to 14 days). This is in agreement with our earlier reports on sucrose metabolism in *S. cerevisiae* (3, 14, 17). It has been reported that under glucose repression, the secretion of internal invertase increases while the external invertase remains low and practically constant (12). The transport of sucrose directly into the yeast cells could possibly explain the presence of internal invertase in the cell vacuoles as earlier suggested by Santos et al. (20). The intracellular enzyme maltase (α -glucosidase) is reported to hydrolyse sucrose (11). However, in the presence of high concentrations of glucose, the maltase enzyme and maltose transport system are repressed (10). It is, therefore, unlikely that sucrose was utilised through the maltose transport system.

The major overall metabolic parameters of growth are presented in Table 1.

Table 1. Metabolic parameters of aerobic growth of *Saccharomyces cerevisiae* 248 UNSW 703100 on mixtures of sucrose and glucose in batch culture after adaptation on sucrose (unless indicated otherwise).

Growth substrate (% w/v)	Adaptation time (days)	μ	Y_{xs}	Q_s	Y_e	Q_e
1% S ^a	2	0.38	0.16	14.0	45.0	17.1
	14	0.55	0.16	19.0	40.0	22.0
1% G ^b	2	0.45	0.15	17.0	41.0	19.0
	14	0.45	0.15	18.0	57.0	26.0
0.5% S + 0.5% G	2	0.45	0.15	17.2	35.5	16.1
	14	0.45	0.13	18.4	44.4	20.0
1% S + 1% G	2	0.45	0.15	16.7	54.7	24.6
	14	0.44	0.13	18.8	41.3	18.2
0.8% S + 1.2% G	2	0.45	0.13	18.8	62.3	28.0
	14	0.47	0.13	20.1	46.9	22.0

^a From Orlowski and Barford (17).

^b From Orlowski and Barford (16). Cells were adapted on glucose.

μ =specific growth rate (h^{-1}), Y_{xs} =biomass yield ($\text{g-biomass g-sugar}^{-1}$), Q_s =specific sugar uptake rate ($\text{mmol equiv. glucose g-biomass}^{-1} \text{h}^{-1}$), Y_e =ethanol yield ($\text{mmol g-biomass}^{-1}$), Q_e =specific ethanol production rate ($\text{mmol ethanol g-biomass}^{-1} \text{h}^{-1}$). S, sucrose; G, glucose.

When the cells are growing on sucrose alone, the specific growth and sugar uptake rates increased from 0.38 to 0.55 h^{-1} and the specific sugar uptake increased from 14 to 19 $\text{mmol g-biomass}^{-1} \text{h}^{-1}$ when the adaptation period was prolonged from 2 to 14 days (17). This is also true for the uptake of radiolabelled sucrose when the cells are adapted on sucrose for a long time (14). When the cells are growing on sucrose and glucose mixtures, the specific growth rate is on average 0.45 h^{-1} for unadapted cells. This is the specific growth rate when the cells are growing on glucose alone (16). However, when the adapted cells were growing on mixtures containing 12 g l^{-1} glucose at inoculation, there was a slight but significant increase in the specific growth rate from 0.45 to 0.47 h^{-1} and the specific sugar uptake rate from 18.8 to 20.1 $\text{mmol glucose equivalent g-biomass}^{-1} \text{h}^{-1}$ (Table 1). These increases could be attributed to the simultaneous uptake of sucrose and glucose (Fig. 2b) during growth rather than the slow hydrolysis of sucrose (limiting levels of invertase). The absence of fructose in the medium could be as a result of either the low sucrose hydrolysis rate (limited by low invertase levels) which caused the fructose resulting from the hydrolysis to be transported into the cell immediately or the cells had the ability to transport sucrose molecules directly without first hydrolysing them. The first alternative seems unlikely since the specific sugar uptake rate for the adapted cells growing on sucrose and glucose mixtures are almost as high as when the cells are growing on sucrose alone (Table 1); it is also higher than that of cells growing on glucose and fructose mixture (17).

The biomass yields ranged from 0.13 to 0.16 $\text{g-biomass g-sugar}^{-1}$ which correspond to those earlier reported in chemostat cultures at high dilution rates (2, 18) and in batch cultures at high sugar concentrations (6, 16). The ethanol yields,

oxygen uptake rates, carbon dioxide production rate and respiratory quotient (RQ) show that the growth was both fermentative and respiratory. At high glucose concentrations, in an aerobic environment, glucose is predominantly fermented despite the availability of oxygen (Crabtree effect) (1, 6). The results in Table 1 for cells growing on the mixtures show that there were high yields of ethanol during growth of the yeast cells on a mixture of glucose and sucrose. This means that the growth of the cells was both respiratory and fermentative, especially during the second part of exponential phase. The specific oxygen uptake rates and carbon dioxide production rates depend on the respiratory capacity of the yeast cells. The respiratory capacity is reported to be affected by the adaptation of the cells on sugars (1). The results shown in Table 1 indicate there was not much difference between the gaseous exchange rates of both the unadapted and adapted states of the cells. This could be that the Crabtree effect (presence of glucose) outweighed the effect of the adaptation process.

Regulation of invertase secretion

When the yeast cells are growing on sucrose alone, the adaptation process does not significantly increase the specific activity of invertase (Table 2). The activity averaged 2.82–3.14 U mg-dry wt⁻¹ after adaptation on sucrose for 2 and 14 days. During early or mid-exponential phase, the cells were mostly utilising glucose and external invertase secretion was repressed. During the late exponential or early stationary phase, the glucose concentration was less than 2 g l⁻¹ and the cells were growing mostly on sucrose. This was accompanied by an abrupt de-repression of invertase secretion. The specific invertase activity in the medium increased from 0.15 to 1.38 U mg-dry wt⁻¹ and from 0.27 to 1.66 U mg-dry wt⁻¹ for cells adapted

Table 2. Invertase regulation by *Saccharomyces cerevisiae* 248 UNSW 703100 growing on mixtures of sucrose and glucose in batch fermenter under fully defined aerobic conditions.

Growth substrate (% w/v)	Adaptation time (days)	Specific invertase activity (U mg-dry wt ⁻¹)	
		E ^a	L ^b
1% S ^c	2	2.82	3.05
	14	2.84	3.14
2% G ^d	2	0.06	0.24
	14	0.26	1.62
0.5% S+0.5% G	2	0.27	1.26
	14	0.15	1.66
1% S+1% G	2	0.18	1.38
	14	0.17	0.70
0.8% S+1.2% G	2	0.12	0.37
	14		

^a Early to mid-exponential phase of growth.

^b Late exponential to early stationary phase of growth.

^c Cells grown on sucrose in shake flasks.

^d Cells adapted on glucose in shake flasks.

S, sucrose; G, glucose. Specific invertase activities in intact cells are an average from three different determinations. The cells were adapted on sucrose unless indicated otherwise.

for 2 and 14 days, respectively (Table 2). However, when the initial glucose concentration in the mixture at inoculation was greater than 10 g l^{-1} (Fig. 2), after the glucose was consumed, there was no resumed secretion of invertase and there was no observed hydrolysis of sucrose. This is confirmed by the absence of fructose (Fig. 2) and by the low levels of invertase in the medium (Table 2). Therefore, there seems to be a correlation between the transport of sucrose directly into the yeast cells and levels of extracellular invertase secreted by the cells. When the cells were growing on glucose, the specific invertase activity was as low as $0.06\text{ U mg-dry wt}^{-1}$ which shows how effectively glucose represses invertase secretion in this strain when present at concentrations above 1% (w/v). This regulation in secretion of external invertase by high concentrations of glucose has been reported before (9, 10, 13). The major important finding in our results is that, for fully sucrose-adapted cells, even in the presence of a repressive glucose concentration, the yeast cells are able to utilise sucrose, and most importantly, without first being hydrolysed to its constituent monosaccharides. Previously, it has been thought that the direct transport of sucrose contributes only a small part of the process of utilisation of sucrose, but as our results indicate, it can be the major mechanism of sucrose utilisation in *S. cerevisiae*.

Mechanism of sucrose utilisation

Traditionally, sucrose is believed to be hydrolysed outside the cells and the hydrolytic products transported across the membrane into the cells of *S. cerevisiae*. This theory alone cannot explain some of the results we have reported in this paper. These results agree with the sucrose-modified cell model proposed earlier (4) in which sucrose utilisation is composed of contributions from both transport of sucrose molecules and its hydrolysis products into the cell. Depending on the conditions in the medium, the two mechanisms can take place concurrently. Hydrolysis of sucrose outside the cell by extracellular invertase is favoured by: (i) glucose concentrations below repressive levels, (ii) high specific external invertase activity, and (iii) unadapted cells. The possibility of direct transport of sucrose is enhanced if: (i) invertase activity is limiting (low), (ii) the cells are fully adapted, and (iii) initial glucose concentrations are high. From these observations, it can be concluded that, in some strains of *S. cerevisiae*, the mechanism by which sucrose is utilised combines both the hydrolysis of sucrose extracellularly and direct transport of sucrose molecules into the cell. It should be noted that the absolute values of the limiting invertase activity and repressive glucose concentration vary from strain to strain of *S. cerevisiae*.

CONCLUSIONS

The ability of a particular strain to transport directly or to hydrolyse sucrose extracellularly is closely related to the levels of invertase activity of the cells, the state of adaptation and the glucose concentration in the medium.

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REFERENCES

- 1) Barford, J. P. and Hall, R. J., An examination of the Crabtree effect in *Saccharomyces cerevisiae*: The role of respiratory adaptation. *J. Gen. Microbiol.*, **114**, 267–275 (1979).
- 2) Barford, J. P., Johnston, J. H., and Mwesigye, P. K., Continuous culture study of transient behaviour of *Saccharomyces cerevisiae* growing on sucrose and fructose. *J. Ferment. Bioeng.*, **79**, 158–162 (1995).
- 3) Barford, J. P., Mwesigye, P. K., Phillips, P. J., Jayasuriya, D., and Blom, I., Further evidence of direct uptake of sucrose by *Saccharomyces cerevisiae* in batch culture. *J. Gen. Appl. Microbiol.*, **39**, 389–394 (1993).
- 4) Barford, J. P., Phillips, P. J., and Orlowski, J. H., A new model of uptake of multiple sugars by *Saccharomyces cerevisiae* (Part 2). *Bioprocess Eng.*, **7**, 303–307 (1992).
- 5) Cason, D. T., Reid, G. C., and Gatner, E. M. S., On the differing rates of fructose and glucose utilisation in *Saccharomyces cerevisiae*. *J. Inst. Brew.*, **93**, 23–25 (1987).
- 6) Copella, S. J. and Dhurjati, P., A detailed analysis of *Saccharomyces cerevisiae* growth kinetics in batch, fed-batch and hollow-fibre bioreactors. *Chem. Eng. J.*, **41**, B27–B35 (1989).
- 7) D'Amore, T., Russell, I., and Stewart, G. G., The effect of carbohydrate adjuncts on brewer's wort fermentation by *Saccharomyces uvarum* (*carlesbergensis*). *J. Inst. Brew.*, **95**, 333–336 (1989).
- 8) Gancedo, J. M., Carbon catabolite repression in yeast. *Eur. J. Biochem.*, **206**, 297–313 (1992).
- 9) Gascon, S. and Lampen, J. O., Purification of the internal invertase of yeast. *J. Biol. Chem.*, **243**, 1561–1572 (1968).
- 10) Görts, C. P. M., Effect of glucose on the activity and kinetics of the maltose-uptake system and of α -glucosidase in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta*, **184**, 299–305 (1969).
- 11) Khan, N. A., Zimmermann, F. K., and Eaton, N. R., Genetic and biochemical evidence of sucrose fermentation by maltase in yeast. *Mol. Gen. Genet.*, **123**, 43–50 (1973).
- 12) Meyer, J. and Matile, Ph., Regulation of isoenzymes and secretion of invertase in baker's yeast. *Biochem. Physiol. Pflanzen.*, **166**, 377–385 (1974).
- 13) Mormeneo, S. and Sentandreu, R., Regulation of invertase synthesis by glucose in *Saccharomyces cerevisiae*. *J. Bacteriol.*, **152**, 14–18 (1982).
- 14) Mwesigye, P. K. and Barford, J. P., Transport of sucrose by *Saccharomyces cerevisiae*. *J. Ferment. Bioeng.*, **77**, 687–690 (1994).
- 15) Neumann, N. P. and Lampen, J. O., Purification and properties of yeast invertase. *Biochemistry*, **6**, 468–475 (1967).
- 16) Orlowski, J. H. and Barford, J. P., The effect of inoculum preparation on the fully aerated growth of *Saccharomyces cerevisiae* with a glucose substrate. *J. Gen. Appl. Microbiol.*, **33**, 113–121 (1987).
- 17) Orlowski, J. H. and Barford, J. P., Direct uptake of sucrose by *Saccharomyces cerevisiae* in batch and continuous culture. *J. Gen. Appl. Microbiol.*, **37**, 215–218 (1991).
- 18) Postma, E., Verduyn, C., Scheffers, W. A., and van Dijken, J. P., Enzymic analysis of the Crabtree effect in glucose-limited cultures of *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.*, **55**, 468–477 (1989).
- 19) Romano, A. H., Microbial sugar transport systems and their importance in biotechnology. *Trends Biotechnol.*, **4**, 207–231 (1986).
- 20) Santos, E., Rodriguez, L., Elorza, M. V., and Sentandreu, R., Uptake of sucrose by *Saccharomyces cerevisiae*. *Arch. Biochem. Biophys.*, **216**, 652–660 (1982).