ELSEVIER

Thermochimica Acta 250 (1995) 233–245

thermochimica acta

Catabolic capacity of *Saccharomyces cerevisiae* in relation to the physiological state and maintenance requirement ☆

Annika Nilsson, Christer Larsson, Lena Gustafsson *

Department of General and Marine Microbiology, Medicinaregatan 9C, Lundberg Laboratory, University of Göteborg, S-413 90 Göteborg, Sweden

Received 15 September 1994; accepted 30 September 1994

Abstract

The importance of the physiological state for the catabolic capacity during carbon- and energy-starving conditions was studied. Endogenous metabolism was low in cells depleted of carbon and energy source. This does not necessarily mean that these cells do not have the capacity for a higher catabolic activity. To measure the catabolic capacity of starved cells, carbon- and energy-depleted cells were suspended in synthetic fresh water and the respiratory and fermentative rates were examined after addition of glucose. The catabolic capacity was studied in cells of different physiological states. Stationary phase cells, which were depleted of their carbon and energy source for 6 h, showed a lower respiratory capacity, but almost as high a fermentative capacity as cells originating from the logarithmic phase of growth on glucose. With extended starvation time of stationary phase cells, the fermentative capacity decreased, while the respiratory capacity increased. Transition phase cells, characterised by a metabolic shift from a mixed respiratory-fermentative catabolism to a purely respiratory catabolism, showed, when newly harvested, a lower fermentative capacity than log phase cells with a mixed respiratory-fermentative catabolism. However, the fermentative capacity decreased during starvation of carbon and energy source for log phase cells, whereas transition phase cells increased the fermentative capacity to the same level as that of newly harvested log phase cells after 4 days without carbon or energy source.

The addition of glucose to stationary phase cells under non-growth conditions (no nitrogen source) resulted in ATP production rates of between 50% and almost 100% of the

* Corresponding author.

☆ Presented at the Ninth Conference of the International Society for Biological Calorimetry, Berlin-Schmerwitz, 27–31 May 1994, and dedicated to Ingolf Lamprecht on the occasion of his 60th birthday.

SSDI 0040-6031(94)02142-2

ATP produced by newly harvested log phase cells under the same conditions. Much higher rates of ATP production were attained for these different types of physiological states than can be explained by maintenance energy requirements. The suggested explanation for this over-consumption of energy during non-growth conditions in response to energy excess is metabolic uncoupling. This behaviour is discussed in relation to maintenance energy requirements during different environmental conditions.

Keywords: Catabolic capacity; *Saccharomyces*

1. Introduction

In his classical, mathematical description of bacterial maintenance energy requirements, Pirt [1] divided substrate consumption into the portion used for growth-associated reactions and the constant portion used for maintenance, which is growth-rate independent. It was later suggested that the maintenance portion includes two components one independent and the other dependent on the growth rate (for a review, see Ref. [2]). However, a unifying mathematical description has not yet been presented (for a discussion, see Refs. [3] and [4]). This may reflect the fact that we still need a better understanding of what, precisely, constitutes the maintenance energy requirements of micro-organisms.

The functions that are generally considered to be included in maintenance energy requirements are those needed for maintaining cellular homeostasis, such as the turnover of macromolecular components and the preservation of the intracellular ionic composition. The maintenance energy demand, however, can be anything between zero and one hundred per cent of the total metabolic energy turnover, depending on external circumstances. The minimum maintenance energy demand during optimal growth is often considered negligible in yeasts (for a discussion, see for example, Ref. [5]). In contrast, during growth under conditions of extreme external stress, the maintenance energy requirement may exceed the maximum amount of energy that can possibly be diverted to maintenance functions. Such a situation will prevent further growth and may even result in cell death, see, for example, Ref. [6]. High maintenance energy demands have, for instance, been demonstrated for yeasts during growth at low extra cellular pH [6,7] and low osmotic potential [8,9]. During non-growth conditions, the total metabolic energy flux may be very small compared to growth conditions and may also under such conditions be completely used for maintenance purposes. However, whether the metabolic energy flux is largely and solely used for maintenance purposes during non-growth conditions depends on which nutrient is limiting for growth. The situation is very different for a culture that is limited by its carbon and energy source compared to limitations such as nitrogen, sulphur or phosphate. In an ammonium-starved culture of *Saccharomyces cerevisiae*, Lagunas and Ruiz [10] determined the costs of known biosynthetic and maintenance processes to be as

little as 15% of the total ATP produced. These data point to uncoupling between anabolism and catabolism during conditions of energy excess compared to other limiting nutrients, an event that has been reported also to occur during growth of both bacteria and yeasts [2,11–13]. Why do mico-organisms under these situations seemingly over-consume energy and, if this is the case, by which mechanism is energy dissipated in these energy substrate-sufficient cultures where catabolism is extensively dissociated from anabolism?

The aim of our work is to understand energy metabolic stratgies of yeasts in response to environmental changes and to be able to differentiate between minimum energy requests for survival and growth in comparison with situations where the energy consumption is vastly greater than would be required to meet the cells' minimum biosynthetic and bioenergetic demands. The present study was performed to elucidate the importance of the physiological state for the cells response towards energy or nutrient limitation during non-growth conditions.

2. Material and methods

2.1. Yeast strain, medium and growth conditions

S. cerevisiae (Baker's yeast strain, Jästbolaget AB, Sweden) was maintained on YPD agar and cultured during experiments in yeast nitrogen base without ammonium sulphate and amino acids (YNB, Difco) supplemented with 5 g l^{-1} (10 g l^{-1} in precultures) of glucose. This medium was further supplemented with 5 g l^{-1} ammonium sulphate (carbon-starvation experiments) or with 0.3 g l^{-1} ammonium sulphate where indicated (nitrogen-starvation, experiments). The experiments were conducted in a 4-l fermentor (1601 Ultroferm; LKB, Sweden). The working volume was 3 l. The temperature was 30°C, the stirring rate 300 rpm and the aeration rate 250 l h^{-1}. The pH was kept constant at 4.5 by automatic addition of 1 M NaOH. Sampling was performed during three specific physiological states: during logarithmic growth on glucose, during the transition phase, and during the stationary phase. Log and transition phase samples were starved for different periods of time according to the starvation protocol (see below), before being tested for their catabolic activity. Stationary phase cells were starved of carbon and energy source (or nitrogen source, where indicated) in their cultivation medium for different periods of time and were subsequently tested for their catabolic capacity.

2.2. Microcalorimetry

The heat production rate (dQ/dt) was measured with a multichannel microcalorimeter (Thermal Activity Monitor LKB 2277; Thermometric AB, Järfälla, Sweden) equipped with flow-through cells. The effective volume of the flow-through cell was, with the flow rate used, 0.43 ml. The effective volume was determined by a chemical calibration using a solution with a known heat production [14].

2.3. Starvation protocol

Cell samples of appropriate volume were washed twice in 0.9% NaCl solution. The cells were suspended in 30 ml of YNB without amino acids, but supplemented with ammonium sulphate (5 g l^{-1}) at the final concentration of 1×10^8 cells per ml. The cell suspensions were thereafter carbon and energy starved for different periods of time on a rotary shaker at 30°C.

2.4. Measurement of catabolic capacity

The cells were harvested by centrifugation and washed twice with L16 ($NaHCO_3$, 84 mg l^{-1}; $NaNO_3$, 12.75 mg l^{-1}; $CaCl_2 \cdot 2H_2O$, 14.70 mg l^{-1}; $MgSO_4 \cdot 7H_2O$, 12.32 mg l^{-1}; K_2HPO_4, 1.74 mg l^{-1}; $Na_2SiO_3 \cdot 9H_2O$, 1.14 mg l^{-1}). The pellet was resuspended in L16 and transferred to a 100-ml E-flask which was incubated at 30°C. Glucose (10 g l^{-1}; final concentration) was immediately added and samples were taken at selected time intervals for determination of ethanol concentrations. In the experiments presented in Figs. 3–5, below, the cell suspension was connected to a flow-through cell of the microcalorimeter before addition of glucose (10 g l^{-1}, final concentration). When a stable heat production rate was obtained, 1 or 10 mM (final concentration) azide was added in order to inhibit respiration. The difference in heat production rate before and after azide addition represents respiratory heat production. The specific oxygen consumption rate can be calculated according to

$$dO_2/dt = (dQ/dt)/\Delta H_{O_2} \quad (1)$$

where dO_2/dt is the specific oxygen consumption rate (mmol g^{-1} h^{-1}), dQ/dt is the difference in specific heat production rate before and after azide addition (J g^{-1} h^{-1}) and ΔH_{O_2} is the enthalpy change accompanying consumption of 1 mmol oxygen (468.9 J $mmol^{-1}$).

When a stable heat signal was obtained after addition of azide, iodoacetic acid (10 mM, final concentration) was added in order to inhibit glycolysis and thus also fermentation. The specific ethanol production can be calculated according to Eq. (2)

$$dEtOH/dt = (dQ/dt)/\Delta H_{EtOH} \quad (2)$$

where $dEtOH/dt$ is the specific ethanol production rate (mmol g^{-1} h^{-1}), dQ/dt is the difference in heat production before and after addition of iodoacetic acid (J g^{-1} h^{-1}) and ΔH_{EtOH} is the enthalpy change accompanying formation of 1 mmol ethanol (50 J $mmol^{-1}$). The small amounts of glycerol and acetate were neglected in these calculations.

2.5. Dry weight determinations

Samples (2×5 ml) were centrifuged for 5 min at $3500 \times g$, washed twice with distilled water, dried for 24 h at 110°C and stored in a desiccator before weighing.

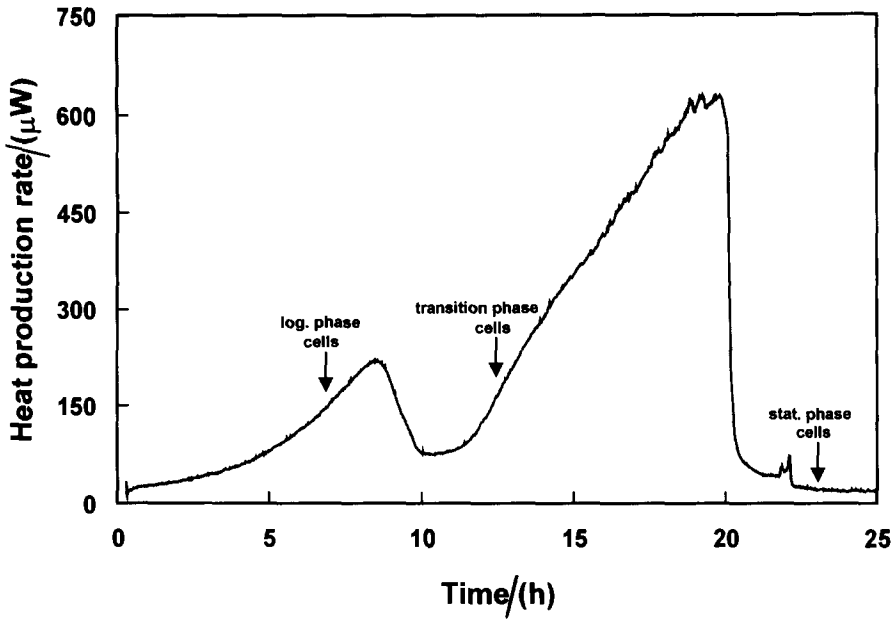

Fig. 1. Changes in heat production rate during aerobic growth of *S. cerevisiae* with 5 g l^{-1} glucose as carbon and energy source and 5 g l^{-1} ammonium sulphate as nitrogen source. The effective volume of the measuring cell was 0.43 ml.

2.6. Measurements of extracellular concentrations of ethanol

Samples (2 × 1.5 ml) were centrifuged for 1 min at 15 000 × ***g***. The supernatants were frozen until analysed, using enzyme combination kits (Biochemica Test Combination; Boehringer Mannheim GmbH, Germany).

2.7. Calculations of ATP production

The fermentative ATP production was calculated from the measurements of ethanol, glycerol and acetate production rates. One mole of ethanol or acetate produced yields one mole of ATP formed whereas each mole of glycerol produced gives a consumption of one mole of ATP. The respiratory ATP production was calculated from the oxygen consumption rates. The calculations are based on a P/O ratio of 0 and 2, respectively. With a P/O ratio of 0 there is 4/6 mole ATP formed per mole O_2 consumed (by substrate level phosphorylation) and a P/O ratio of 2 gives 4.67 mole ATP formed per mole O_2 consumed.

3. Results and discussion

A typical pattern of the rate of heat production during aerobic batch cultivation of *Saccharomyces cerevisiae* with glucose as the sole carbon and energy source is

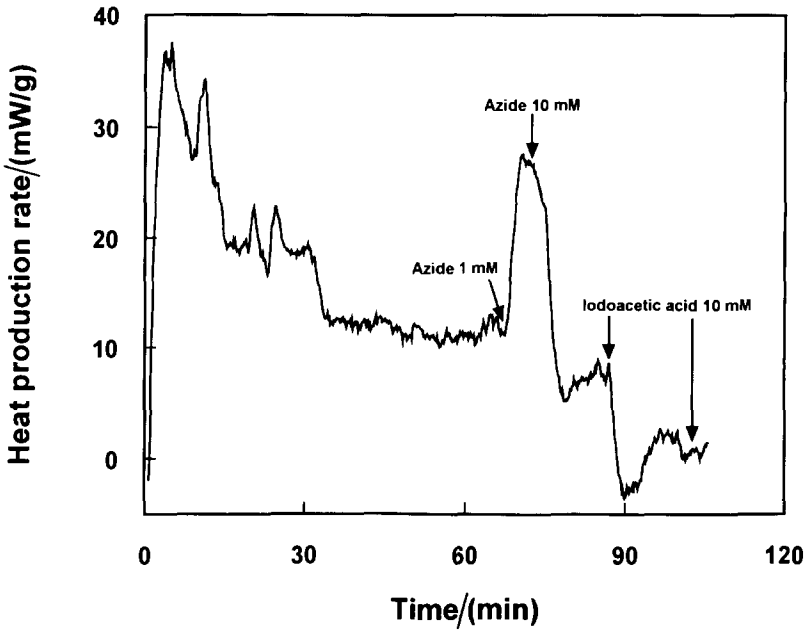

Fig. 2. Changes in specific heat production rate of stationary phase cells (depleted of both carbon and energy sources as well as nitrogen source) of *S. cerevisiae* after transfer to synthetic fresh water (L16) and subsequent additions of azide and iodoacetic acid.

shown in Fig. 1. At the first maximum of the heat production rate, glucose became limiting, which resulted in a shift from a mixed respiratory-fermentative catabolism to a purely respiratory catabolism. During the following phase, ethanol (together with small amounts of acetate and glycerol) produced during the respiro-fermentative phase was respired. Depletion of carbon resulted in a sharp drop in the rate of heat production, back to the baseline, demonstrating the entrance of the stationary phase (for more details, see Refs. [15] and [16]). Cells of three different physiological states (Fig. 1) were tested for their catabolic capacity: log phase cells, growing with a mixed respiratory-fermentative catabolism; transition phase cells, just entering the respiratory phase by using mainly ethanol as the carbon and energy source for growth; and stationary phase cells, depleted of all external carbon and energy sources. (Two different ammonium sulphate concentrations were used, 0.3 and 5.0 g l^{-1}, respectively. With the lower concentration, stationary phase cells were not only depleted of carbon and energy sources but also of the nitrogen source).

3.1. Endogenous metabolism

Stationary phase cells, persisting in their growth medium (depleted of external carbon and energy sources or depleted of carbon, energy and nitrogen sources), showed no measurable metabolic activity, as judged by calorimetric measurements.

If, however, cells from such cultures were transferred to a synthetic fresh-water salt solution (L16), an increased metabolic activity was triggered (Fig. 2). This increased activity was due to consumption of endogenous energy sources. After an initial burst of activity, the rate of heat production stabilised at about 10 mW g^{-1}, i.e. the endogenous metabolic activity corresponded to 36 J g^{-1} h^{-1}. Both respiratory and fermentative catabolism contributed to this metabolic activity, because azide (10 mM, final concentration) and iodoacetic acid (10 mM, final concentration) both partly reduced the metabolic activity (Fig. 2). A lower final concentration of azide (1 mM) triggered the cells to an even higher level of metabolic activity. This indicates that cells are also able to regulate their metabolic activity when using endogenous metabolism.

3.2. Catabolic capacity

To study the catabolic capacity of cells originating from the three different physiological states outlined in Fig. 1, cells were harvested and resuspended in synthetic fresh-water (L16) and subsequently given a glucose pulse. The respiratory capacity was determined from the difference in heat production rate before and after addition of azide (Figs. 3 and 4), which inhibits respiration. The fermentative capacity was determined in a similar way, but in this case iodoacetic acid was used

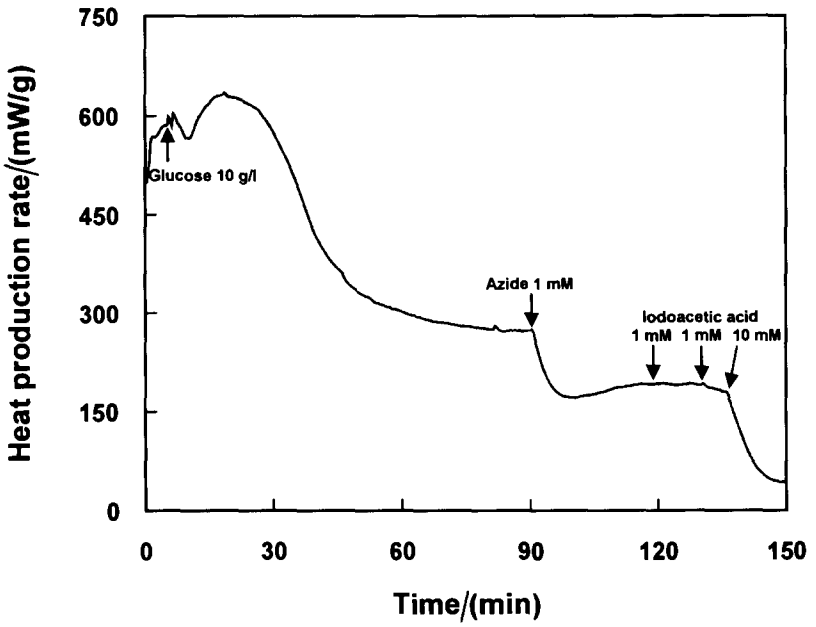

Fig. 3. Changes in specific heat production rate of stationary phase cells (depleted of carbon and energy sources) of *S. cerevisiae* after transfer to synthetic fresh water (L16) and subsequent additions of glucose, azide and iodoacetic acid.

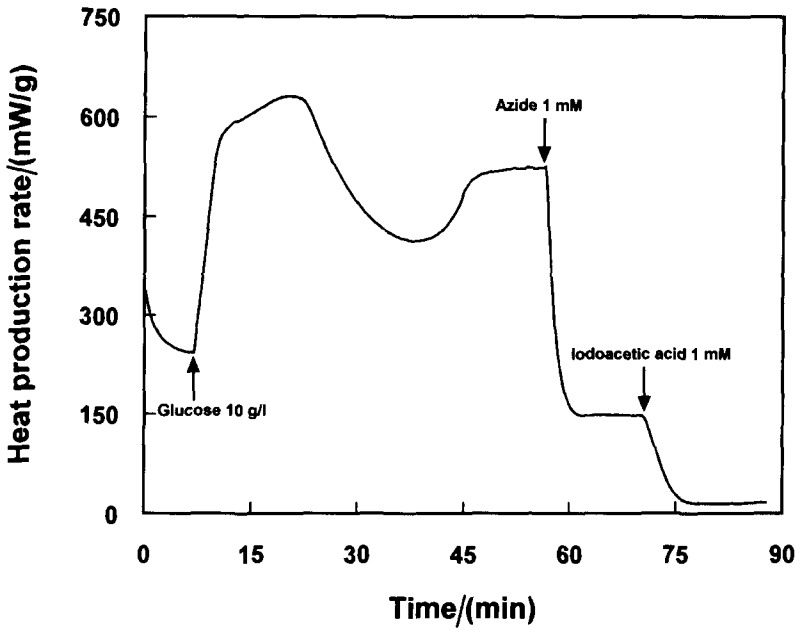

Fig. 4. Changes in specific heat production rate of log phase cells (logarithmic growth on glucose) of *S. cerevisiae* after transfer of synthetic fresh water (L16) and subsequent additions of glucose, azide and iodoacetic acid.

in order to inhibit glycolysis (Figs. 3 and 4) and thereby fermentation. The fermentative capacity measurements were verified by direct enzymatic measurements of ethanol production, whereas the respiratory measurements have not yet been verified with a direct method.

When comparing the heat production rate data in Fig. 3 with those presented in Fig. 4, one realises that stationary phase cells starved of carbon and energy still have a high fermentative as well as respiratory capacity. In a recent report [17], in which the catabolic capacity of carbon- and nitrogen-starved cells were compared, the respiratory capacity of stationary phase cells (starved of carbon and energy for 6 h) was calculated as one third of the respiratory capacity of log phase cells (see Figs. 3 and 4). The values attained were 1 mmol and 3 mmol of oxygen consumption per g biomass and h, respectively. The fermentative capacity was almost the same in the log phase and stationary phase cells, i.e. about 12 mmol of ethanol produced per g dry biomass and h (see Figs. 3 and 4). With increased time of carbon and energy starvation, the fermentative capacity decreased slowly, while the respiratory capacity increased. By assuming a P/O ratio varying from 0 to 2, the respiratory and fermentative ATP production was calculated from the data presented by Larsson et al. [17] (Fig. 5). By assuming the same P/O ratio for the different physiological states, the total ATP production in stationary phase cells (starved of carbon and energy for 6 h) attained 65%–95% of that produced in log

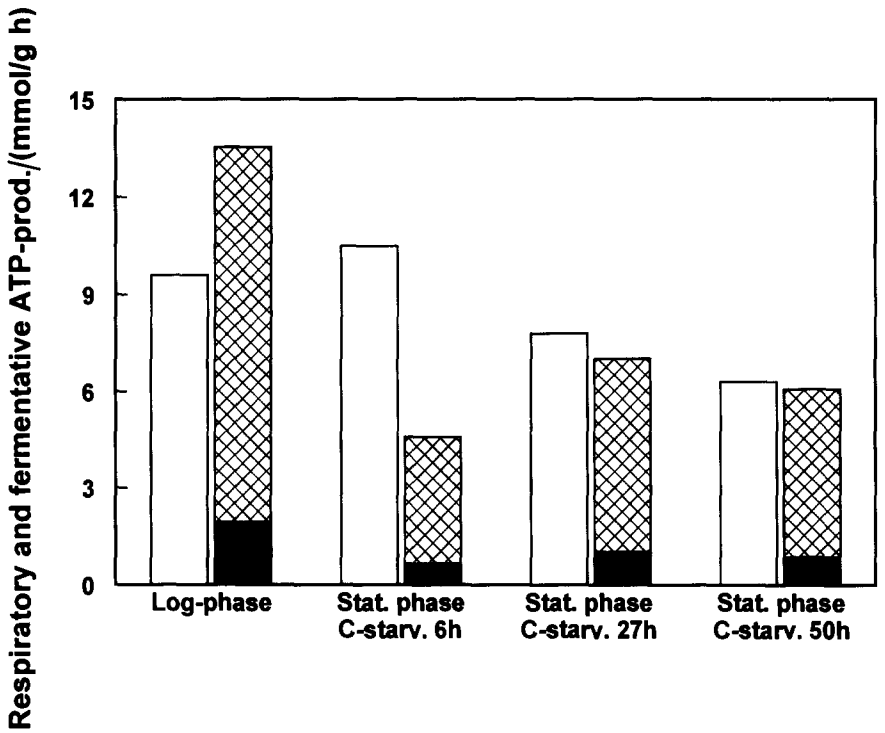

Fig. 5. Fermentative and respiratory ATP production of log phase cells (logarithmic growth on glucose) and stationary phase cells (depleted of carbon and energy sources for 6, 27 and 50 h, respectively) of *S. cerevisiae* after transfer to synthetic fresh water (L16) and subsequent addition of glucose (10 g l^{-1} final concentration). The open columns represent fermentative ATP production. The black and crosshatched columns represent respiratory ATP production by assuming a P/O ratio of 0 and 2, respectively.

phase cells. After 50 h of carbon and energy starvation, the stationary phase cells still had the ability to produce 55%–65% of the ATP produced by log phase cells per unit of time. However, it has to be kept in mind that the values obtained for the log phase cells also represent the rate of ATP production by resting cells (newly harvested and suspended in synthetic fresh-water), when given a glucose pulse. Because almost any biomass production can be excluded, except for a small production of reserve material [10], large amounts of ATP seem to be produced for unknown functions, both by cells originating from the log phase as well as from the stationary phase. Such data can hardly be interpreted in any other way than that *S. cerevisiae* is highly over-consuming the energy source when prevented from growth by limitation of other nutrients, which is in accordance with the data presented by Lagunas and Ruiz [10].

A surprising observation was that stationary phase cells, which are derepressed from glucose repression after growth on a respiratory substrate (ethanol) and also as a result of total carbon and energy depletion during the subsequent stationary

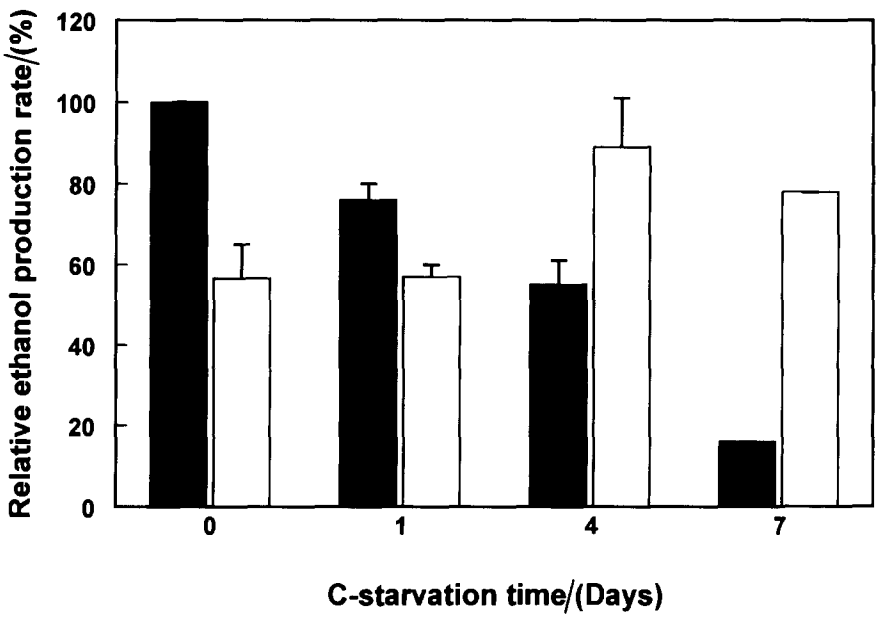

Fig. 6. Relative ethanol production rates of log phase cells (logarithmic growth on glucose, black columns) and transition phase cells (transition from glucose to ethanol as carbon and energy source, open columns) of *S. cerevisiae* after starvation for 0, 1, 4 and 7 days. The cells were harvested and starved in a medium without carbon (YNB without amino acids, Difco) for up to 7 days at 30°C. The ethanol production rate was measured by transferring the cells to a synthetic fresh water solution (L16) and subsequent addition of glucose (10 g l^{-1} final concentration). Two separate experiments were performed and the error bars show the variation between the duplicated experiments.

phase, showed a lower respiratory capacity than cells originating from a logarithmically growing culture on glucose which used a mainly fermentative metabolism with only very limited respiratory rate due to catabolite repression. We also compared the fermentative capacity between log phase cells and transition phase cells. The cells originating from these two physiological states differed in that log phase cells were glucose-repressed, while the transition phase cells were glucose-derepressed. As expected, newly harvested log phase cells showed the highest fermentative capacity (Fig. 6), but this situation was reversed after 1–4 days of starvation of carbon and energy source (all other nutrients were available in excess) at 30°C. At day 4, the fermentative capacity of the transition phase cells was as high as that of newly harvested log phase cells. The rationale for this behaviour is difficult to explain with our present knowledge. The conclusion so far is that the catabolic capacity is highly dependent on the physiological state of the cells, being not only affected by the phase of growth, but also by the starvation time and by the type of limiting substance [17].

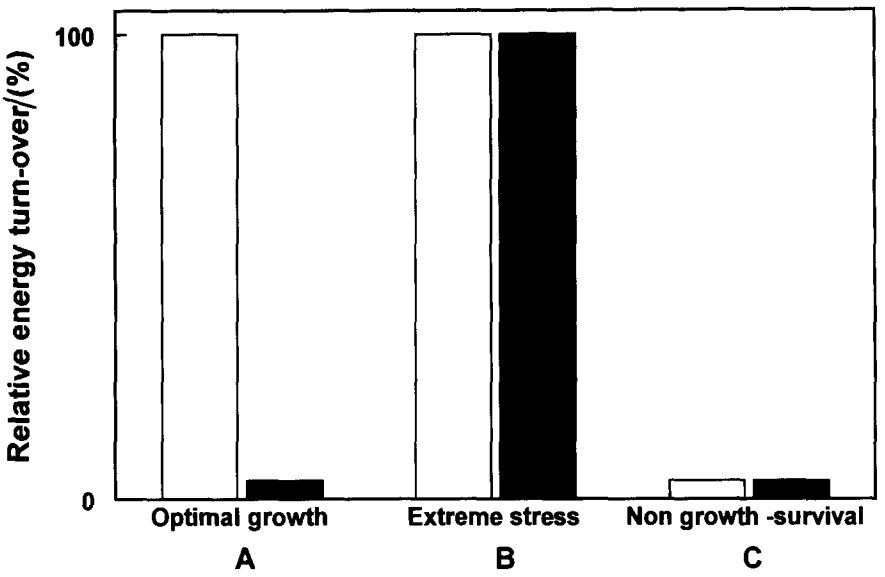

Fig. 7. Hypothetical partitioning between the total metabolic energy turnover (open columns) and the energy used for maintenance functions (black columns) under different conditions.

3.3. Maintenance energy requirements

Four different categories of behaviour in relation to the maintenance energy demand can be distinguished. First, maintenance energy demands during optimal growth conditions are usually considered small and even negligible in comparison with the energy allocated to biosynthetic and other growth-associated processes (Fig. 7A) [6,9]. This partitioning between energy used for growth-associated and for maintenance processes, respectively, is, however, far from clear [4].

Second, maintenance energy demands may increase considerably due to an external stress situation. During such a situation, all or a substantial part of the total energy available can be used for maintenance purposes (Fig. 7B). For example, when *S. cerevisiae* was growing in chemostats at an NaCl concentration of 0.9 M, the additional maintenance energy expenditure was, depending on the growth rate, 14–31% of the total energy requirement for growth at 0 M NaCl. Including the energy conserved in the dominating osmoregulator, glycerol, the total additional energy demand for growth at 0.9 M NaCl corresponded to 28–51% of the energy required for growth at 0 M NaCl [8]. By assuming a P/O ratio of 1.0, the additional maintenance demand imposed by salt stress was calculated to correspond to 2–3 mmol ATP per g and h [18], which is in accordance with a value of 1.7 mmol ATP per g and h for anaerobic chemostats of *S. cerevisiae* cultured in 1 M NaCl [9]. The maintenance energy requirement was as high as 9 mmol ATP per g and h at an external pH of 2.8 during anaerobic chemostat growth of *S. cerevisiae* [6] and the catabolic energy turnover more than doubled during aerobic

batch growth of *Debaryomyces hansenii* at an external pH of 3 compared to that at pH 4.5–6 [7]. Verduyn et al. [6] calculated that the fermentation rate of *S. cerevisiae* limits the supply of ATP for anabolism below a pH of 2.8 where growth stops due to the high maintenance requirements at these extreme situations [6,7].

Third, knowledge of the maintenance energy requirement during non-growth conditions is scarce and may be very difficult to determine, particularly if Bulthuis et al. [4] are correct in their conclusion that the maintenance energy requirements are not constant and, furthermore, probably not linearly correlated with growth rate. The transition from active growth to survival during starvation has been demonstrated to be an active process during which the cells have to rely on endogenous sources (for a discussion see Ref. [19]), while the energy turnover during long-term starvation-survival is probably very low (see above) and not measurable by conventional methods. During starving conditions, the energy available endogenously may be used completely for maintenance purposes (Fig. 7C), including *de novo* synthesis of starvation proteins.

Finally, a totally different situation to that which has been discussed above arises during conditions of nutrient limitation, but with carbon and energy source in excess (see above). The increased energy consumption during such conditions is both for growing and non-growing cells separated from maintenance energy requirements and can instead be referred to as uncoupling of catabolism from anabolism. The questions, "why do micro-organisms consume energy in vastly greater amounts than would be required to meet the cells' minimum biosynthetic and bioenergetic demands?" and "by which mechanism is energy dissipated in these cells where catabolism is extensively uncoupled from anabolism?", still remain unsolved. However, Teixeira de Mattos et al. [20] suggested that uncoupling of metabolism from energy generation is a general feature of microbial metabolism, which indeed is a hypothesis in agreement with our results. These authors [20] also suggested that mechanisms allowing uncoupling of catabolism from anabolism permit the organisms to respond to environmental changes and endow the organisms with metabolic flexibility.

Acknowledgements

This work was supported by grants from the Swedish National Board for Technical Development, Nordic Industrial Fund and from the Swedish baker's yeast manufacturer Jästbolaget AB.

References

[1] S. Pirt, The maintenance energy of bacteria in growing cultures, Proc. R. Soc. London Ser. B, 163 (1965) 224–231.

[2] D. Tempest and O. Neijssel, The status of Y_{ATP} and maintenance energy as biologically interpretable phenomena, Ann. Rev. Microbiol., 38 (1984) 459–486.

[3] S. Pirt, The energetics of microbes at slow growth rates: Maintenance energies and dormant organisms, J. Ferment. Technol., 65 (1987) 173–177.
[4] B. Bulthuis, G. Koningstein, A. Stouthamer and H. van Verseveld, A comparison between aerobic growth of *Bacillus licheniformis* in continuous culture and partial-recycling fermentor, with contributions to the discussion of maintenance energy demand, Arch. Microbiol., 152 (1989) 499–507.
[5] C. Verduyn, A. Stouthamer, W. Scheffers and J. van Dijken, A theoretical evaluation of growth yields of yeasts, Antonie van Leeuwenhoek; J. Microbiol. Serol., 59 (1991) 49–63.
[6] C. Verduyn, E. Postma, W. Scheffers and J. van Dijken, Energetics of *Saccharomyces cerevisiae* in anaerobic glucose-limited chemostat cultures, J. Gen. Microbiol., 136 (1990) 405–412.
[7] L. Gustafsson and C. Larsson, Energy budgeting in studying the effect of environmental factors on the energy metabolism of yests, Thermochim. Acta, 172 (1990) 95–104.
[8] R. Ölz, K. Larsson, L. Adler and L. Gustafsson, Energy flux and osmoregulation of *Saccharomyces cerevisiae* grown in chemostats under NaCl stress, J. Bacteriol., 175 (1993) 2205–2213.
[9] T. Watson, Effects of sodium chloride on steady-state growth and metabolism of *Saccharomyces cerevisiae*, J. Gen. Microbiol., 64 (1970) 91–99.
[10] R. Lagunas and E. Ruiz, Balance of production and consumption of ATP in ammonium-starved *Saccharomyces cerevisiae*, J. Gen. Microbiol., 134 (1988) 2507–2511.
[11] G. Lidén, A. Persson, L. Gustafsson and C. Niklasson, Energetics and product formation by *Saccharomyces cerevisiae* grown in anaerobic chemostats under nitrogen limitation, submitted.
[12] C. Larsson, U. von Stockar, I. Marison, and L. Gustafsson, Growth and metabolism of *Saccharomyces cerevisiae* in chemostat cultures under carbon-, nitrogen-, or carbon- and nitrogen-limiting conditions. J. Bacteriol., 175 (1993) 4809–4816.
[13] C. Larsson, U. von Stockar, I. Marison, and L. Gustafsson, Metabolic uncoupling in *Saccharomyces cerevisiae*, 9th ISBC Conf., Berlin-Schmerwitz, Germany, 1994, S10.
[14] A. Chen and I. Wadsö, A test and calibration process for microcalorimeters used as thermal power meters, J. Biochem. Biophys. Meth., 6 (1982) 297–306.
[15] A. Blomberg, C. Larsson and L. Gustafsson, Microcalorimetric monitoring of growth of *Saccharomyces cerevisiae*: Osmotolerance in relation to physiological state, J. Bacteriol., 170 (1988) 4562–4568.
[16] C. Larsson, A. Blomberg and L. Gutafsson, Use of microcalorimetric monitoring in establishing continuous energy balances and in continuous determinations of substrate and product concentrations of batch-grown *Saccharomyces cerevisiae*, Biotechnol. Bioeng., 38 (1991) 447–458.
[17] C. Larsson, A. Nilsson and L. Gustafsson, Catabolic capacity of carbon- or nitrogen-starved cultures of *Saccharomyces cerevisiae*, in E. Gnaiger, F.N. Gellerich and M. Wyss (Eds.), What is controlling life, Modern Trends in Biothermokinetics, Vol. 3, Innsbruck University Press, 1994, pp. 232–237.
[18] L. Gustafsson, R. Ölz, K. Larsson, C. Larsson and L. Adler, Energy balance calculations as a tool to determine maintenance energy requirements under stress conditions, Pure Appl. Chem., 65 (1993) 1893–1898.
[19] L. Gustafsson, Microbiological calorimetry, Thermochim. Acta, 193 (1991) 145–171.
[20] M. Teixeira de Mattos, H. Streekstra, O. Neijssel and D. Tempest, The Link Between the Efficiency of ATP Generation and Metabolic Flexibility During Anaerobic Growth on Various Carbon Sources, IRL Press, Oxford, Washington, 1987.