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Energetics of the Budding Cycle of *Saccharomyces cerevisiae* during Glucose Limited Aerobic Growth

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Summary. A method for the estimation of the yield on energy (Y_{ATP}) and of the efficiency of oxidative phosphorylation, *in vivo* (P/O ratio) is described, which is based on the measurement of effective gas exchange values (Q_{O_2} and Q_{CO_2}) and of the yield coefficient Y of continuously growing populations of baker's yeast which vary in the degree of fermentation and respiration. For Y_{ATP} a value of 12.0 ± 0.5 $\frac{\text{mg dry weight formed}}{\text{mMole ATP}}$ and for P/O ratio one of 1.1 ± 0.05 $\frac{\text{mMole ATP}}{\text{1/2 mMole } O_2}$ was found and seems to be independent of the type of glucose catabolism (under glucose limitation).

The gas exchange of populations of *Saccharomyces cerevisiae* synchronized at different growth rates was determined. The specific oxygen uptake and carbon dioxide formation rate, Q_{O_2} and Q_{CO_2}, are shown to depend on the state of the cells in the budding cycle. Increase in gas metabolism and therefore increased energy generation coincides with the initiation of budding. The longer the generation time $\bar{g}$ the more expressed are these oscillations of energy formation over the budding cycle. The relationship between the course of energy generation and energy storage and the sequence of budding and single cell phase over the division cycle is discussed.

In previous years several studies dealt with the relation between growth, substrate and oxygen consumption, between growth and energy generation of different microorganisms (Monod, 1942; Bauchop and Elsden, 1960; Senez, 1962; Hadjipertou *et al.*, 1964). Generally anaerobic growth conditions were used (Herandez and Johnson, 1967a) to determine the yield on energy Y_{ATP} (Bauchop and Elsden, 1960) as the more simple experimental procedure did not need analyses of metabolized gases. As well as many bacteria yeasts (*Saccharomyces* sp. and *Candida* sp.) were studied. Anaerobically the yield on energy of *Saccharomyces cerevisiae* at growth on glucose (pure fermentation) was determined to be 10.5 mg dry weight formed/mMole ATP (Bauchop and Elsden, 1960). Under aerobic conditions Y_{ATP} for *Saccharomyces* sp.

and *Candida* sp. could not be definitively calculated (CHEN, 1964; HERNANDEZ and JOHNSON, 1967b) as the efficiency of oxidative phosphorylation (P/O ratio) is not exactly known, either for intact cells (CHANCE, 1959a, b; LYNEN and KOENIGSBERGER, 1951) or for isolated mitochondria (ONISHI *et al.*, 1966). Thus the question about the proportionality between growth and energy generation, about the energetic coupling of catabolism and anabolism and about the constancy and effective value of P/O ratio could not be answered.

In the present paper a calculation of the yield on energy Y_{ATP} for different P/O ratios is performed, based on the measurement of glucose catabolism of baker's yeast by the analysis of metabolized gases (FIECHTER and MEYENBURG, 1968) in function of the specific growth rate μ in aerobic continuous culture with glucose limitation (FIECHTER and MEYENBURG, 1966a, b; MEYENBURG, 1968). This serves to examine the proportionality between growth and energy formation (constancy of Y_{ATP}) and to find the effective P/O ratio for intact growing cells of *Saccharomyces cerevisiae*.

Recently the sequence of budding and single cell phase in the budding cycle of baker's yeast was described to depend on the specific growth rate for glucose limited continuous growth (MEYENBURG, 1968). The budding phase (time between initiation of budding and scission) is rather constant (1.3—2.0 hrs for generation times $\bar{g}$ of 1.3 and 15 hr, respectively) whereas the single cell phase varies widely from zero to 13 hr. It was concluded from these findings that the fulfilment of the budding period in a constant time is made possible by storage of reserves (carbohydrates) during the single cell phase and their breakdown during the bud formation, producing enough energy to enable the mitosis with DNA-replication (WILLIAMSON, 1966), formation of new structures and cell wall material.

Synchronization of baker's yeast populations at low growth rates in continuous culture gave us the possibility to study the course of gas metabolism (O_2 consumption and CO_2 release) and of carbohydrate storage and reactivation (KÜENZI and FIECHTER, 1969) over the division cycle.

Materials and Methods

Organism: *Saccharomyces cerevisiae* (baker's yeast), strain *LBG H 1022*

Growth Conditions

Cells were grown at 30° C in the liquid synthetic medium NL 18: glucose (7% water), 30 g; $(NH_4)_2SO_4$, 7.5 g; KH_2PO_4, 1.5 g; $MgSO_4 \cdot 7\,H_2O$, 0.5 g; $CaCl_2 \cdot 6\,H_2O$, 0.06 g; $FeSO_4\ (NH_4)_2SO_4 \cdot 6\,H_2O$, 12 mg; $ZnSO_4 \cdot 7\,H_2O$, 6 mg; $CuSO_4 \cdot 5\,H_2O$, 1 mg; m-inosite, 60 mg; thiamine, 14 mg; pyridoxine, 3 mg; Ca-pantothenate, 1.5 mg; d-biotine, 0.1 mg; glutamic acid, 2.25 g; yeast extract "Difco", 0.75 g: aqua dest. ad 1,000 ml. Glucose-vitamine and mineral salt solutions

were sterilized separately and combined afterwards. In this medium glucose is the limiting substrate ($S_O = 28$ g/l). The cultivations were performed in a bench scale fermentor (Chemap AG., Männedorf, Switzerland; cf. FIECHTER, 1965) under aerobic conditions with controlled pH at 5.5, as described by BECK and MEYENBURG (1968). The air flow rate of 2.5 vvm and mixing by flat blade turbines at 900 rpm gave a sufficiently high oxygen transfer rate (135 mMoles O_2/l per hour). *Continuous cultivation* was carried out according to the chemostatic principle (NOVICK and SCILARD, 1950). The working volume V was 2.55 l at a medium flow rate $F = 100$ ml/hr and increased linearly to 2.85 l at $F = 1{,}400$ ml/hr. At different dilution rates, $D = F/V$, the cell population was grown to steady states ($\rightarrow$ specific growth rate $\mu \equiv D$) which were analysed for dry weight (dw), glucose, ethanol concentration, (FIECHTER and MEYENBURG, 1966a) and gas exchanges (FIECHTER and MEYENBURG, 1968). The yield coefficient Y, specific oxygen uptake Q_{O_2} and carbon dioxide release Q_{CO_2} of the growing population were calculated from these data.

Synchronization

Synchronization of the yeast population was carried out at different growth rates in the oxidative range of aerobic growth in continuous culture (at dilution rates between zero and 0.24). Synchronous budding was obtained by simple shift-down in dilution rate ($D_1 \rightarrow D_2 = {}^1/_2 D_1$) or by periodic shift-up ($\rightarrow D_1$) and shift-down ($\rightarrow D_2$) according to the mean generation time ($g = 0.69/\bar{D}$, where $\bar{D}$ is between D_1 and D_2). The method will be described in more detail (MEYENBURG, 1969). Synchronization for experiments at minimal generation time was performed by separation of a random population (from first growth phase in batch culture; MEYENBURG, 1968; MEYENBURG and FIECHTER, 1966) by dextrin gradient centrifugation. Cells of the late stage of budding phase could be isolated from the others due to their low specific density and were subsequently used as synchronous inoculum. This method was developed by A. WIEMKEN (cf. WIEMKEN *et al.*, in press).

The synchrony was controlled by determination of the population distribution according to the state of the cells in the budding cycle (MEYENBURG, 1968). The percentage (frequency) of the initial budding cells IBC ($\sim$ mitotic index) was used as base for the calculation of the degree of synchrony (ENGELBERG, 1964).

Results and Discussion

A. Characteristics of Aerobic Growth of *Saccharomyces cerevisiae* Under Glucose Limitation in Continuous Culture

The growth of *Saccharomyces cerevisiae* in continuous culture in NL 18 (see Fig. 1) is distinguished by a range of purely oxidative metabolism ($RQ = 1.0$ for dilution rates from 0.0—0.24) and a range of increasing fermentative type of glucose catabolism. In the former range a linear increase of the specific gas exchange values Q_{O_2} and Q_{CO_2} was determined (cf. HERBERT, 1958). This results in proportionality to specific growth rate μ if corrected for the gas exchange for the energy generation for maintenance, $Q_{O_2}m = Q_{CO_2}m = 0.62$ mMole O_2 resp. CO_2/g dw per hour (Table 1). In the latter range a more marked increase for Q_{CO_2} was determined whereas Q_{O_2} decreases constantly due to

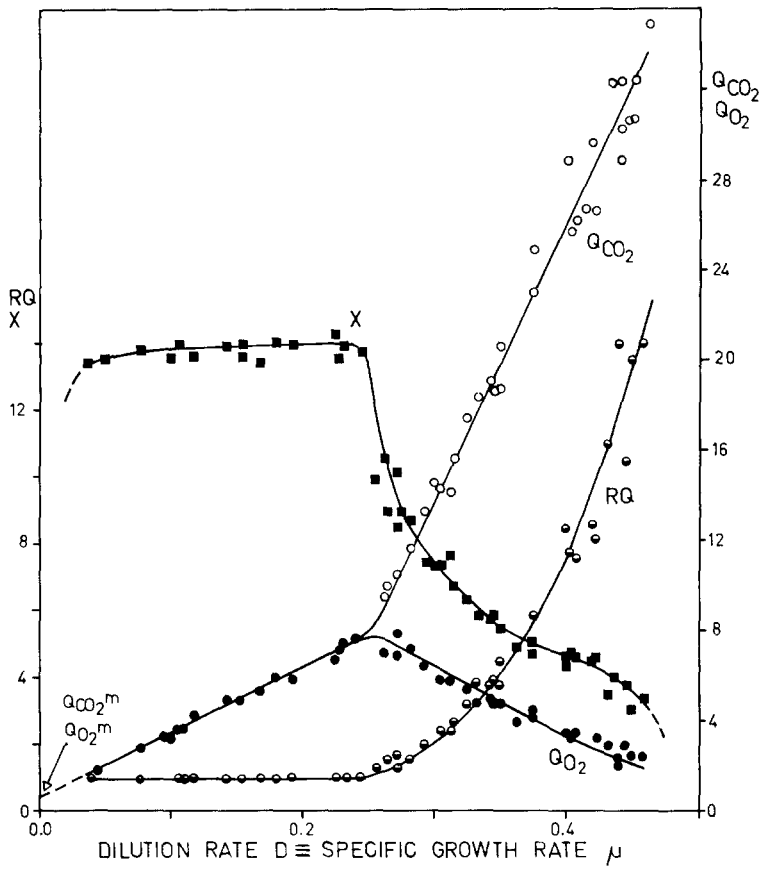

Fig. 1. Aerobic growth of *Saccharomyces cerevisiae H 1022* in continuous culture under glucose limitation. Course of dry weight in the medium X (mg/ml), specific oxygen uptake Q_{O_2} and carbon dioxide production Q_{CO_2} (mMoles/g dw per hour), and of the respiration quotient RQ (Q_{CO_2}/Q_{O_2}) in function of the dilution rate $D \equiv$ specific growth rate μ (hr^{-1}). $Q_{O_2}m$, $Q_{CO_2}m$: Gas exchange for energy generation or maintenance, determined by linear extrapolation of Q_{O_2} and Q_{CO_2} to $D =$ zero

repression of TCA-cycle and respiratory enzymes (BECK and MEYENBURG, 1968) resulting in the increase of RQ from 1.0 (until $\mu = 0.24$) to 13.3 (at $\mu = 0.45\ h^{-1}$). This change in metabolism is paralleled by a decrease of the yield coefficient from 0.50—0.145 caused by the increasing aerobic fermentation of glucose to ethanol and carbon dioxide (Table 1). For detailed discussion of these features see FIECHTER and MEYENBURG (1966a, b); MEYENBURG (1968).

Table 1. *Aerobic growth of* Saccharomyces cerevisiae H 1022 *in continuous culture under glucose limitation. Mean values for yield and gas metabolism derived from the curves in Fig. 1*

Dilutionrate, D = specific growth rate, μ (hr^{-1})	Yield on carbon source, Y $\left(\frac{\text{mg dry weight}}{\text{mg glucose}}\right)$	Q_{CO_2} $\left(\frac{\text{mMoles}}{\text{g dw} \cdot \text{hr}}\right)$	Q_{O_2} $\left(\frac{\text{mMoles}}{\text{g dw} \cdot \text{hr}}\right)$	RQ Q_{CO_2}/Q_{O_2}
0.00	—	0.62[a]	0.62[a]	1.00
0.05	0.483	2.06	2.06	1.00
0.10	0.493	3.53	3.53	1.00
0.15	0.497	4.96	4.96	1.00
0.20	0.500	6.43	6.43	1.00
0.24	0.497	7.60	7.60	1.00
0.275	0.322	10.90	7.32	1.49
0.30	0.264	13.8	6.52	2.11
0.325	0.228	16.8	5.68	2.96
0.35	0.198	20.0	4.88	4.10
0.375	0.180	23.2	4.12	5.6
0.40	0.168	26.3	3.40	7.7
0.425	0.154	29.5	2.91	10.2
0.45	0.145	32.7	2.45	13.3

[a] Extrapolated values of the gas exchange for energy generation for maintenance, $Q_{CO_2}m$ resp. $Q_{O_2}m$.

B. Determination of Y_{ATP}, P/O Ratio, and Q_{ATP} for *Saccharomyces cerevisiae* from Data of Glucose Limited Aerobic Growth

Bauchop and Elsden (1960) assumed that the growth of microorganism is proportional to the "biologically useful energy, to the amount of energy made available by a catabolic process which the cell is able to use for the chemical, osmotic, and mechanical work associated with growth". This proportionality called yield on energy Y_{ATP} may be calculated as the ratio between cell dry weight formed and the amount of energy generated or as ratio between specific growth rate and specific generation of energy used for growth, which is more appropriate to the results gained by continuous culture experiments:

Equation 1

$$Y_{ATP} = \frac{\mu}{Q_{ATP} - Q_{ATP}m}$$

Y_{ATP}: Yield on energy (mg dw formed/mMole ATP).
Q_{ATP}: Specific generation of biologically useful energy at specific growth rate μ (mMoles ATP/g dw per hour).
$Q_{ATP}m$: Specific generation of energy for maintenance (mMoles ATP/g dw per hour).

Q_{ATP} is the sum of the energy generation rates of all energy (ATP) yielding reactions of catabolism of the limiting carbon source. For

aerobic glucose catabolism in *Saccharomyces cerevisiae* there are two main routes of energy formation: 1. via Embden-Meyerhof pathway (glycolysis) by substrate level phosphorylation of which the efficiency is expressed by the P/CO_2 (P/C_3) ratio, 2. via TCA-cycle and oxidative phosphorylation of which the efficiency is expressed by the P/O ratio. The equation for the calculation of Q_{ATP} for *Saccharomyces cerevisiae* from the specific gas exchange values Q_{O_2} and Q_{CO_2} (mMoles/g dw per hour) in continuous culture (i.e. in function of $D = \mu$) consists of four terms:

Equation 2

$$Q_{ATP} = \underset{(1)}{Q_{O_2} \cdot P/O} + \underset{(2)}{{}^1/_3\, Q_{CO_2}ox \cdot P/CO_2} + \underset{(3)}{Q_{CO_2}ferm \cdot P/CO_2} + \underset{(4)}{8.8\, C_3 \cdot P/C_3 \cdot \mu}$$

(1) ATP formation by oxidative phosphorylation.

(2) ATP formation via glycolysis (Embden-Meyerhof pathway) of the amount of glucose which is completely oxidized afterwards through the TCA-cycle.

(3) ATP formation via glycolysis of the amount of glucose fermented to ethanol and CO_2.

(4) ATP formation via glycolysis of the portion of glucose of which the carbon appears in the newly formed dry matter (48% C in dry matter). $^2/_3$ of the cell carbon is assumed to derive from glycolytic pyruvate.

$Q_{CO_2}ox$: Oxidative CO_2 formation $= Q_{O_2}$.
$Q_{CO_2}ferm$: CO_2 formation by aerobic fermentation $= Q_{CO_2} - Q_{CO_2}ox$.
P/O: Efficiency of oxidative phosphorylation (mMoles ATP/$^1/_2$ mMole O_2).
P/CO_2, P/C_3: Efficiency of substrate level phosphorylation (glycolysis; 1.0 mMole ATP/mMole CO_2 resp. C_3).
μ: Specific growth rate (g dw formed/g dw present per hour $= hr^{-1}$).

Energy formation by the pentose-P-shunt is neglected because its participation in the total CO_2 evolution is less than 5% in baker's yeast (CHEN, 1959; KOVACHEVICH and GUZMAN BARRON, 1964).

The rate of energy generation for maintenance is considered to be constant over the whole range of dilution rate (cf. HERBERT, 1958) and is calculated as it follows:

Equation 3

$$Q_{ATP}m = Q_{O_2}m \cdot P/O + {}^1/_3\, Q_{CO_2}m \cdot P/CO_2$$

P/CO_2 (or P/C_3) has a value of 1.0 mMole ATP per mMole C_3 formed ($C_2 + CO_2$, respectively; LYNEN and KOENIGSBERGER, 1951) whereas the value of the P/O ratio is not accurately known. CHANCE (1959) showed the effective P/O value to be about 1 which was near the value obtained by LYNEN and KOENIGSBERGER (1951). Because of this uncertainty Q_{ATP} and subsequently Y_{ATP} is calculated for different P/O ratios from

the Q_{CO_2} and Q_{O_2} values (according to the equation above) at different growth rates (from Table 1) corresponding to different ratios between oxidative and fermentative energy generation. These calculated values

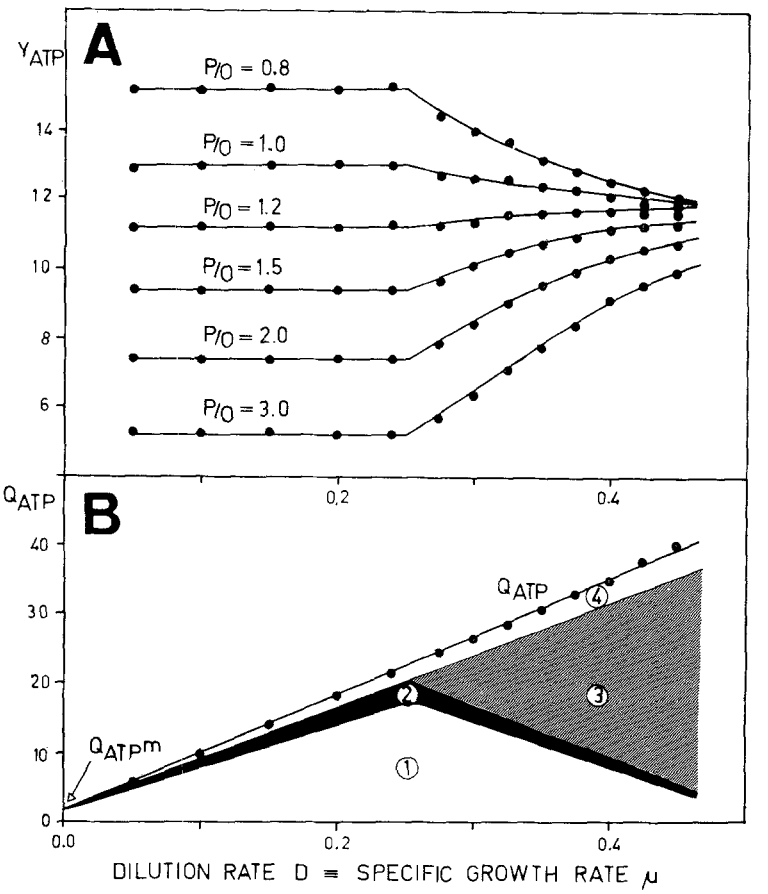

Fig.2A and B. Energetics of the aerobic growth of *Saccharomyces cerevisiae* in continuous culture under glucose limitation. A. Yield on energy Y_{ATP} (mg dw formed/mMole ATP) calculated for various efficiencies of oxidative phosphorylation P/O in function of the dilution rate $D \equiv \mu$. B. Total specific energy generation Q_{ATP} (mMoles ATP/g dw per hour) and specific energy generation by the different pathways for P/O ratio = 1.1 in function of the dilution rate $D \equiv \mu$. The numbers correspond to the ones in Equation 2, indicating the portions of energy formed by:
(1) oxidative phosphorylation
(2) "oxidative glycolysis" } substrate level phosphorylation
(3) "fermentative glycolysis" } substrate level phosphorylation
(4) "synthetic glycolysis" } substrate level phosphorylation
$Q_{ATP}m$: specific energy generation for maintenance = 1.57 mMoles ATP/g dw per hour

for Y_{ATP} are plotted against specific growth rate in Fig. 2A. It is evident that neither the lowest P/O = 0.8 nor the high ones as 2.0—3.0 give constant values for Y_{ATP} in function of μ. From this graph the effective P/O ratio which fulfills the assumption of constancy of Y_{ATP} can be easily determined:

This P/O ratio for growing cells of *Saccharomyces cerevisiae* is 1.1 ± 0.05 mMoles ATP/$^1/_2$ mMole O_2. The yield on energy Y_{ATP} is 12.0 ± 0.5 mg dw formed/mMole ATP.

The linearity for P/O = 1.1 between μ and Q_{ATP}—which is the sum of the energy contributed by the different catabolic reactions (1—4) —is shown in Fig. 2B. This proves at the same time the proportionality between energy generation (after correction for $Q_{ATP}m = 1.57$ mMoles ATP/g dw per hour) and growth, i.e. the constancy of Y_{ATP}, and the constancy of the efficiency of oxidative phosphorylation (P/O) for different growth rates and for different types of glucose catabolism at glucose limited growth of *Saccharomyces cerevisiae*. The P/O ratio found by this method based on the analysis of continuous growth is in accordance with the values found by LYNEN and KOENIGSBERGER (1951) and CHANCE (1959b) who measured the rates of phosphorylation and dephosphorylation. The relatively low efficiency of oxidative phosphorylation, although three active sites of phosphorylation were determined (CHANCE, 1959a), is due to the high rates of dephosphorylation (CHANCE, 1959b). The constancy of P/O deduced from the evident linearity of Q_{ATP} in function of μ for P/O = 1.1 (Fig. 2B) indicates a fixed balance between dephosphorylation and phosphorylation.

The value of 12.0 for Y_{ATP} is somewhat higher than the values reported by BAUCHOP and ELSDEN (1960) and HERNANDEZ and JOHNSON (1967b). This is possibly due to the method of continuous culture which was used to study fully balanced cell populations. It is supposed that in energetic studies with batch cultures "adaptation" effects (e.g. changing control of enzyme synthesis resulting in derepression; see BECK and MEYENBURG, 1968) were not completely excluded, which gives rise to higher energy consumption for the shifts in the cellular network.

Considering the Y_{ATP} of 12 to be also correct for other yeasts we can calculate the effective P/O ratio e.g. for *Candida* sp. from the reported yields on oxygen Y_{O_2} (WHITAKER and ELSDEN, 1963; HERNANDEZ and JOHNSON, 1967b). This yield coefficient was shown to be 1.25—1.32 mg dw/mg O_2 which is contrary to the determined values for *Saccharomyces cerevisiae* of 0.92 (by HERNANDEZ and JOHNSON, 1967b) and 0.98 for the range of oxidative growth in continuous culture (present study). From that a P/O ratio for *Candida* sp. of 1.5—1.6 mMoles ATP/$^1/_2$ mMole O_2 could be calculated. The higher growth yields of this yeast (CHEN, 1964; HERNANDEZ and JOHNSON, 1967b) might be explained by this

higher efficiency of oxidative phosphorylation, while the more oxidative type of growth must be due to changed control patterns concerning the enzymes. The calculation of Y_{ATP} for the oxidative growth of *Saccharomyces cerevisiae* on ethanol at a specific growth rate of 0.20 h^{-1} for P/O ratio = 1.1 gave values in the range of 6.8—7.2 indicating that growth on ethanol is less efficient. This is probably due to the necessary formation by the gluconeogenetic pathway of hexosephosphates for polysaccharide and nucleotide synthesis and activation by ATP of each molecule of ethanol after oxidation to acetate and before the entry to the TCA-cycle and glyoxylate bypass. This is a three times higher "loss of energy" for the activation of substrate than with glucose.

C. Energy Generation over the Budding Cycle of *Saccharomyces cerevisiae* at Glucose Limited Aerobic Growth

The basis analysis of synchronous growth of baker's yeast at a generation time $\bar{g} = 9.5$ h — obtained by a shift-down in dilution rate from 0.15 h^{-1} to 0.07 — are compiled in Fig. 3. About 20 min before the initiation of budding can be determined microscopically (increase in the percentage of initial budding cells, % IBC), a very marked increase of the carbon dioxide production Q_{CO_2} and a lower one of the oxygen consumption Q_{O_2} was detected, followed by a 10% decrease in dry weight X. The respiration quotient RQ increases to slightly fermentative values (1.6—1.8) at the time of the sudden rise of gas metabolism paralleled by an increase in the ethanol concentration A. After this sudden change of metabolic rates, which is to be considered as the initiation I of the budding, RQ falls to values below 1.0 ($\rightarrow$ 0.85), showing the oxidation of the previously accumulated ethanol. According to the degree of synchrony and growth rate (cf. Fig. 4) gas metabolism remains increased during 1.0—1.5 hrs. After the initial peak the course of Q_{CO_2} exhibits a second and a third one. On the other hand after the first increase the course of Q_{O_2} shows a slower constant one and decreases as Q_{CO_2}— when ethanol concentration fell below 15 γ/ml—to values similar to those before the sudden increase.

The length of budding phase from initiation of budding (I) until scission of the daughters from the mother cells (S) is two hours and is followed by a 7.5 hrs single cell phase before the onset of the next budding period. This is in good agreement with the earlier finding of the independence of the length of budding phase in the growth cycle of *Saccharomyces cerevisiae* on the specific growth rate (Meyenburg, 1968). The present results give evidence for the hypothesis that the cells make possible the accomplishment of the formation of the bud in a constant time by the storage of reserves during the single cell phase and their

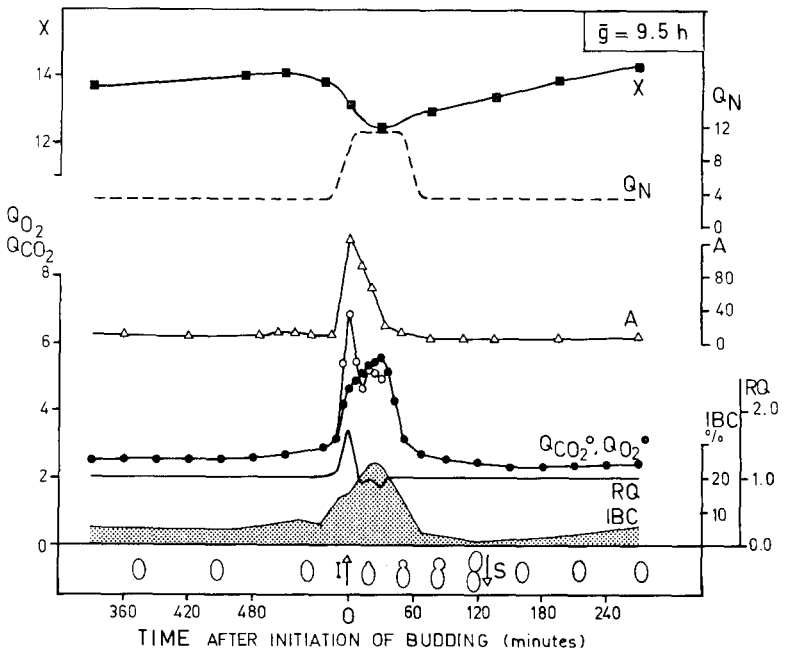

Fig. 3. Synchronous growth of *Saccharomyces cerevisiae* under glucose limitation in continuous culture at a mean generation time $\bar{g} = 9.5$ hr ($D = 0.073$). Course of dry weight in the medium X (mg/ml), specific oxygen uptake Q_{O_2} and carbon dioxide production Q_{CO_2} (mMoles O_2 resp. CO_2/g dw per hour), respiration quotient RQ, percentage of initial budding cells (% IBC), ethanol concentration in the medium A (γ/ml), and specific rate of nitrogen uptake Q_N (mg N/g dw per hour) over the budding cycle. *I* Initiation of budding, *S* Scission of the daughter from the mother cell

breakdown during the budding. The slight increase of dry weight in the medium during the single cell phase and the decrease at the initiation of budding indicates the accumulation of some cell constituents and its fast reactivation. Recently it was shown by KÜENZI and FIECHTER (1969) that these reserves are carbohydrates, namely trehalose and glycogen. The short increase of RQ to fermentative values above 1.0 supports the view that stored carbohydrates are the additional substrates, the breakdown of these giving rise to Q_{O_2} and Q_{CO_2} at the initiation of budding. The rate of energy generation is proportional to the rate of gas metabolism as it follows from the energetics of asynchronous growth of *Saccharomyces cerevisiae*. Thus, the increase of Q_{ATP} enables the cells to form the new bud, nucleic acids and proteins at a higher velocity over the budding period as can be seen from the course of specific nitrogen uptake rate Q_N (Fig. 3).

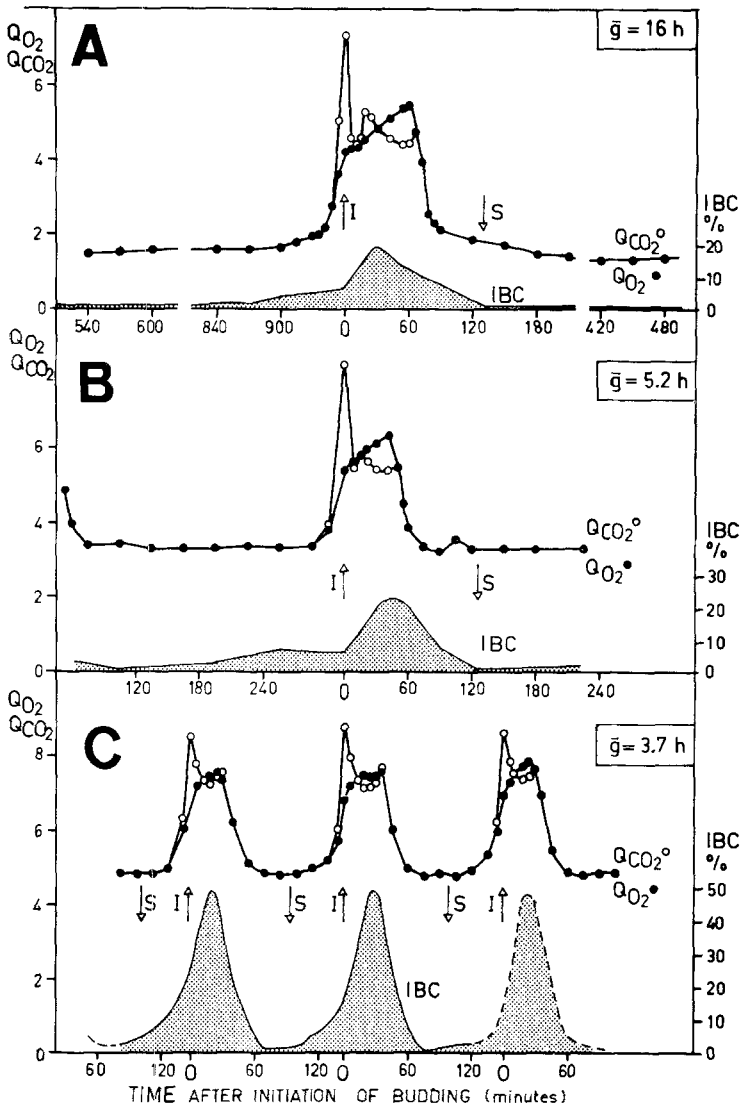

Fig. 4 A—C. Synchronous growth of *Saccharomyces cerevisiae* under glucose limitation in continuous culture at different generation times. Course of specific gas exchanges Q_{O_2} and Q_{CO_2} (mMoles O_2 resp. CO_2/g dw per hour) and of the percentage of initial budding cells (% IBC) over the budding cycle. *I* Initiation of budding, *S* Scission of the daughter from the mother cells. A. Mean generation time $\bar{g} = 16$ hr, corresponding to $D = 0.045$ hr^{-1}. B. Mean generation time $\bar{g} = 5.2$ hr, corresponding to $D = 0.133$ hr^{-1}. C. Mean generation time $\bar{g} = 3.7$ hr, corresponding to $D = 0.185$ hr^{-1}. The stable synchrony oscillations have a period of 2.5 hr and were followed over more than twenty cycles without any decrease of the amplitude. A discussion on the problem of the stabilization of oscillations due to synchronous growth will be published elsewhere (MEYENBURG, in press)

Logically, it can be concluded that the longer the generation time—i.e. the lower the rate of substrate feeding and therefore of energy generation—the changes in gas metabolism over the budding cycle are more

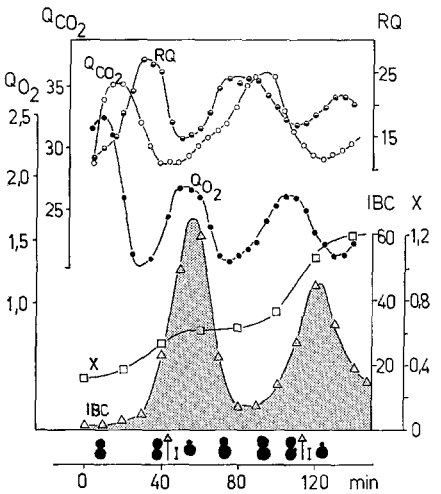

Fig. 5. Synchronous growth of *Saccharomyces cerevisiae* in 3% glucose medium as batch culture with a mean generation time $\bar{g}$ of 1.3 hr ($\mu_{max} = 0.53$ hr^{-1}). Course of the specific gas exchanges Q_{O_2} and Q_{CO_2} (mMoles O_2 resp. CO_2/g dw per hour), the respiration quotient RQ, and the percentage initial budding cells (% IBC) over the budding cycle. *I* Initiation of budding (Scission takes place after or in the moment of the initiation of the next budding period)

Table 2. *Synchronous growth of* Saccharomyces cerevisiae *under various degrees of glucose limitation. Synchronization index SI and mean, minimal, and maximal values of* Q_{ATP} *(for* $P/O = 1.1$*) over the budding cycle at different mean generation times* $\bar{g}$

mean generation time $\bar{g}$ (hr)	$D \equiv \mu$	SI %	specific energy generation (mMoles ATP/g dw per hour)		
			mean Q_{ATP}	minimal Q_{ATP}	maximal Q_{ATP}
16	0.045	45	5.50	4.4	15.5
9.5	0.073	30	7.85	6.7	15.7
5.2	0.133	33	13.5	9.7	18.0
3.7[a]	0.185	46	19.5	13.8	22.5
1.3[b]	0.53	41	41.0	38.2	43.3

[a] Stable synchrony oscillations with a period of 2.5 hr, see MEYENBURG (in press).

[b] Synchronous growth of baker's yeast as batch culture. Synchronization index refers to the first cell cycle.

expressed due to the storage of carbohydrates to higher concentration during the more expanded single cell or maturation phase. This is clearly demonstrated in Fig. 4 A, B and C. The lower the generation time the less impressive are the changes of the energy generation ($\sim Q_{O_2}$, Q_{CO_2}) at the onset of budding. But even at minimal generation time of 1.3 hr at full aerobic fermentation (RQ 15—25; glucose concentration 3%) neither Q_{O_2} and Q_{CO_2} (cf. WILLIAMSON, 1964) nor RQ are constant over the cell cycle (Fig. 5), nor is the increase of dry weight steady but shows stepwise increase (this latter is in contrast to the findings of WILLIAMSON, 1964). It led to the suggestion that even under excess glucose there is a timing of the energy generation at the expense of reserves. In Table 2 the extreme values of specific energy generation over the budding cycle are compiled proving the relationship between generation time and the intensity of the change of Q_{ATP}. The reserve carbohydrate content of asynchronous populations of baker's yeast was shown earlier to increase at low growth rates (FIECHTER and MEYENBURG, 1966b). This supports the present explanations on energy storage and activation in function of the growth rate over the growth cycle.

The question whether the concentration of reserve carbohydrates, or the activity of certain enzymes, or the concentration of metabolites, or the initiation of DNA-synthesis, is the primary factor which induces the mobilization of the stored energy can not be answered at the present time. I can only point to the extreme importance of this timed and timing energy generation over the cell cycle for the control of the overall growth process of the individual cell. In recent experiments (MEYENBURG, 1969) the concentration of various metabolites (e.g. adenosine phosphates, pyridine nucleotides) was shown to vary in correlation with the changes of Q_{ATP}. This may give the possibility to explain the timing of enzyme synthesis (GORMAN *et al.*, 1964; TAURO and HALVORSON, 1966) by division cycle inherent catabolite repression (BECK and MEYENBURG, 1968).

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