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ORIGINAL PAPER

Substrate inhibition kinetics of *Saccharomyces cerevisiae* in fed-batch cultures operated at constant glucose and maltose concentration levels

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Abstract Fed-batch culture is the mode of operation of choice in industrial baker's yeast fermentation. The particular mode of culture, operated at stable glucose and maltose concentration levels, was employed in this work in order to estimate important kinetic parameters in a process mostly described in the literature as batch or continuous culture. This way, the effects of a continuously falling sugar level during a batch process were avoided and therefore the effects of various (stable) sugar levels on growth kinetics were evaluated. Comparing the kinetics of growth and the inhibition by the substrate in cultures grown on glucose, which is the preferential sugar source for *Saccharomyces cerevisiae*, and maltose, the most common sugar source in industrial media for baker's yeast production, a milder inhibition effect by the substrate in maltose-grown cells was observed, as well as a higher yield coefficient. The observed sugar inhibition effect in glucostat cultures was taken into account in modeling substrate inhibition kinetics. The inhibition coefficient K_i increased with increasing sugar concentration levels, but it appeared to be unaffected by the type of substrate and almost equal for both substrates at elevated concentration levels.

Keywords *Saccharomyces cerevisiae* · Fed-batch culture · Glucostat · Substrate inhibition kinetics · Glucose · Maltose

List of symbols

K_S	cell growth saturation coefficient (g l^{-1})
K_i	inhibition coefficient (g l^{-1})
S	substrate (sugar) concentration (g l^{-1} or mM)
t	time (h)
q_S	specific sugar uptake rate (μM s^{-1} g CDW^{-1})
Y	yield coefficient
μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})
x	biomass (g l^{-1})

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Introduction

Saccharomyces cerevisiae is able to utilize a wide range of mono-, di- and oligosaccharides as well as respiratory substrates such as ethanol, acetic acid, pyruvate, lactate and glycerol. Glucose is the favorite carbon source and the preferred mode of metabolism is the fermentative, using the EMP pathway and resulting in the formation of ethanol [10]. The typical fermentation is anaerobic, but *S. cerevisiae* is a facultative anaerobe and can respire on low sugar concentrations or while using respiratory substrates [10, 47].

Yeast cell membranes are not freely permeable to highly polar sugar molecules and various complex mechanisms exist for the transport of glucose and other saccharides into the cell [7]. *S. cerevisiae* is very efficient at hexose transport. It possesses 19, or possibly 20, genes encoding hexose transporters [7, 23, 30]. These transporters differ in their abundance and intrinsic affinities for hexoses [30]. It is also possible that at the very high sugar concentrations encountered in industrial fermentation media, simple diffusion may account for a proportion of uptake into yeast cells [48]. Concerning glucose transport in *S. cerevisiae*, it is well-known so far that there is no singular, common way, in which the yeast transports glucose. There is a high-affinity system, which is absent in cells growing in high [c. 2% (w/v)] levels of glucose [6]. Under these conditions, low-affinity glucose transport systems are operable, which are constitutive and independent of phosporylation [6, 7, 47]. Glucose uptake exhibits differential kinetics depending on the extracellular availability of glucose. The general consensus is that under non-steady-state growth conditions, for example, during batch culture growth [11], there is a biphasic or occasionally multiphasic catalytic uptake exhibited by high- and low-affinity carriers. The former is characterized by a relatively low transport affinity constant, K_T, of around 1 mM glucose and the latter by a higher K_T of around 20 mM glucose. In comparison with other yeasts, these K_T values for *S. cerevisiae* are uncharacteristically high [44, 47].

Whereas glucose and galactose are translocated in *S. cerevisiae* by facilitated diffusion, disaccharides generally enter by concentrative active proton-symport mechanisms [47]. Maltose is the disaccharide, which is the most abundant fermentable sugar present in barley malt extracts for brewer's, distiller's and baker's yeast applications. Maltose uptake may be a rate-limiting determinant in such fermentations. *S. cerevisiae* transports maltose by an energy-dependent proton-symport mechanism and hydrolyses the sugar by an intracellular maltase to two molecules of glucose. It appears in a number of reports that maltose transport is performed by an inducible, high-affinity (K_T 4 mM) and a constitutive, low-affinity (K_T 70 mM) component operable under high maltose concentrations [23]. The latter component, however, was attributed to an experimental artifact and the transport activity is due not to a genuine transport process but to non-specific binding of maltose to cell wall and/or plasma membrane [23]. In industrial strains, there is evidence for the existence of a low-affinity maltose transporter [39] and this is not unusual, since industrial strains clearly differ from laboratory strains with regard to maltose uptake characteristics. The high-affinity maltose transporter in *S. cerevisiae* is induced by maltose, repressed by high (>0.4%, w/v) glucose and inactivated following growth on glucose or by nitrogen exhaustion [12, 13, 40, 42]. This transporter has been characterized both genetically and biochemically. In addition to the gene of the specific transporter (maltose-specific carrier or permease), maltose utilization requires the products of two other genes, maltase and an activator of transcription [39, 49].

Saccharomyces cerevisiae is the prototypical Crabtree-positive yeast [8]. To ensure optimal growth, aerobic conditions must be maintained in the reactor. Sugar is then oxidized into CO_2 and H_2O to generate the energy required for growth and metabolism. Under anaerobic conditions, the growth rate is reduced, more sugar is consumed and ethanol is produced. Ethanol can also be produced in the presence of sugar in large amounts [43, 45]. As with oxygen, an increase of the sugar concentration is followed by a higher growth rate, but beyond a certain point a further increased sugar concentration causes production of ethanol. The growth rate continues to increase but on account of higher sugar consumption and with a considerable lower acceleration [21]. This pattern is similar to that of anaerobic growth, even though the oxygen supply may be sufficient. To obtain a high yield of biomass on sugar, it is necessary to keep the sugar concentration below the point where ethanol production is initiated. This point of sugar concentration is called the ''critical level'' and this is the reason why the process of baker's yeast production is operated in fed-batch mode in the industry [21]. The particular mode of operation allows a successive addition of the sugar, thus avoiding the high initial sugar concentrations of a pure batch operation.

Baker's yeast is a typical low-value, high-volume product with well-established fed-batch procedures being the mode of operation [21]. The manufacturers' specific know-how is mainly concerned with quality control with the result that the information on specific production aspects freely available is somewhat limited. *S. cerevisiae's* preference for glucose is well-known. On the other hand, the main types of sugar substrates used in the industrial applications of *S. cerevisiae* are the α-glucosides (maltose, maltotriose and sucrose) with maltose being the most representative [4, 9, 35]. Although the uptake and transport kinetics of glucose and maltose by yeast cells has been the subject of a large number of studies, the majority of these have been performed in batch or chemostat cultures and have been focused on biochemical and genetic analysis of sugar transport. Recently, attempts to improve

maltose fermentation efficiency in brewing and baking yeasts have been undertaken [16, 19, 20]. However, yeast growth kinetics and modeling in fed-batch cultures operated at constant sugar concentration levels have not been reported earlier. In view of the above, in this work, we report on a detailed kinetic analysis of an industrial baker's yeast strain grown in fed-batch mode operated as glucostat (a stable sugar level is maintained throughout the process) on glucose, and similarly on maltose. Operating in such a mode, we avoid the effects of an ever-changing sugar level during a batch process and we monitor the effects of a stable sugar level on growth kinetics.

Materials and methods

Microorganism and inoculum preparation

The industrial strain (baker's yeast) *S. cerevisiae* EL1 (property of the University of Strathclyde) was used throughout this study. This was streaked onto MPYG agar surface and the plates were incubated at 30°C for 2 days. Then, a single colony from a plate was transferred aseptically to a 500-ml Erlenmeyer flask containing 100 ml of fermentation medium. Three flasks were used as inoculum for bioreactor cultures. The flasks were incubated in an orbital shaker incubator (SANYO Gallencamp, SANYO Biomedical Europe BV, UK) at 200 rpm at 30°C for 12 h.

Culture conditions

The stirred tank bioreactor used in this work was a BIOSTAT ED-ES10, B. Braun Biotech International, with a working volume of 5–10 l. The internal diameter of the culture vessel was 0.22 m and the height-to-diameter ratio 3:1. The agitation system consisted of three disk turbine impellers, each with six flat blades. The pH was controlled with addition of titrants [2 M NaOH and 10% (w/w) H_2SO_4] at 5.5. Process temperature was maintained at 30°C and the airflow rate at 1 volume/volume/min (vvm). Foam control was by addition of polyethylene glycol mol wt 3,500 by Sigma (St Louis, MO, USA) prior to sterilization.

The media used in this study were the following: (1) MPYG Medium (Peptone Yeast Extract Glucose Medium, Modified). The original formulation contains 5 g l^{-1} glucose. With the addition of glucose to the original formulation, the medium used in this study contained glucose in the range of 5–100 g l^{-1} (27.7–555.5 mM). (2) GYP Medium (Glucose Yeast Extract Peptone Medium). The original formulation contains 10 g l^{-1} glucose. The medium in our study was made to contain glucose in the concentration range of 2–10 g l^{-1}. (3) MPY Broth (Maltose Peptone Yeast Extract Broth). The original formulation contains 2 g l^{-1} maltose. Maltose was added to the standard formulation in the concentration range of 5–100 g l^{-1} (13.8–277.5 mM). Sugars were from Sigma (St Louis, MO, USA), while all other medium components were from Difco Laboratories. Standard medium formulations were as described by Atlas [1].

Batch fermentations' working volume was 10 l. In glucostat cultures, the initial volume was 9 l and the final (after addition of feed) was 10 l. For fed-batch experiments, all ingredients of the start-up medium were prepared for 10 l of medium, except for the sugar of interest (glucose or maltose), which was prepared for 9 l of medium with the remaining added in the feed. One liter of sugar solution was fed to keep the sugar concentration in the culture stable for an overall of 12 h. Sugar concentration in the solution and the feed rate (ml h^{-1}) were adjusted to keep the sugar level stable during the period of interest. The concentrations of sugar feed in each run depended on the rate of sugar consumption, which was estimated from batch experiments. Feeding started with the completion of the lag phase (2 h after inoculation). All runs terminated at the 15th hour from inoculation. Fermentations were carried out in triplicates and mean values are reported here.

Analytical methods

Dry-weights were determined by filtering 10 ml of fermentation broth through pre-weighed glass fiber filters (grade GF/C, 4.25 cm, Whatman International, Maidstone, UK), washing and drying in a microwave oven for 15 min at low power and keeping in a dessicator for 24 h before re-weighing. Glucose was determined by the method of Kunst et al. [22]. Maltose concentrations were determined using the Maltose UV assay kits by Boehringer (Mannheim, Germany). Ethanol was determined using the Ethanol UV assay kits by Boehringer (Mannheim, Germany). The ATP content of cells was determined according to Zhong et al. [51].

Calculation methods for μ_{max}, K_S, Y and q_S

Calculations of the above kinetic parameters were made by using the Monod equation $\mu = \frac{\mu_{max} S}{S + K_S}$ (for μ_{max}), the Lineweaver–Burk plot (for K_S), the equation $Y = x/S$ for batch experiments and $x/S_i - S_0$ (for the yield coefficient, Y), where S is the concentration of

carbon source. The specific glucose uptake rate q_S is the quotient of growth rate/yield (in millimolar sugar per g CDW per g S). The inhibition coefficient K_i was determined by using the equation $\mu = \frac{\mu_{max} S}{K_S + S + S^2/K_i}$ according to Jackson and Edwards [14]. Definitions of kinetic parameters and calculations were according to Sinclair and Cantero [38], and Sinclair [37]. The modeling software ModelMaker 4 was used for determination of kinetic parameters values from experimental data. The Monod equation and the above equation by Jackson and Edwards [14] were used for the simulating model to fit experimental data and optimize these parameters.

Results and discussion

A series of batch fermentations were carried out for the whole range of glucose and maltose concentrations applied, as described in the previous section, for the estimation of sugar consumption rates. Fermentations were performed under fully aerated conditions and the dissolved oxygen levels were always above 65% of saturation. Within the range of sugar concentration of 5–100 g l^{-1} (glucose or maltose) biomass concentrations obtained at the end of runs (15 h from inoculation) were from 1.35 to 8 g l^{-1} for glucose media and from 2.3 to 7 g l^{-1} for maltose media. In Fig. 1, a typical glucostat experiment is given, performed at 10 g l^{-1} glucose concentration. The feeding strategy for the glucostat cultures was based on the calculated rates from batch experimental data.

Figure 2 shows the plot of various kinetic parameters' values in glucostat cultures with glucose used as the sugar source. Glucose concentrations are given in the millimolar form. The plot shows that the level of glucose concentration influences growth-kinetics by. Maximum specific growth rate values increase sharply with increasing glucose level up to the point of 55.5 mM to decline steadily beyond that point. This can be explained by the inhibition of the enzymes involved in respiratory metabolism even at saturated oxygen levels at elevated sugar levels. This catabolite repression of respiratory enzyme synthesis (Crabtree effect) leads to respiro-fermentative growth and reduction of μ_{max}. q_S increased up to the concentration level of 277.7 mM glucose while a sharp drop was observed beyond that point. The cell growth saturation coefficient K_S increased, while the yield coefficient Y decreased with increasing glucose concentration levels. The numerical value of K_S reflects the microorganism's affinity for its substrate. A high K_S value is indicative of low affinity and a low K_S value is indicative of high affinity. These results suggest that the assimilation of the substrate-glucose is influenced by its level of concentration and a possible reason for the observed decline in μ_{max} values at high glucose concentration levels would be the elevated K_S values at these levels. The relationship between the yield coefficient Y and glucose concentration levels can be approximated to be

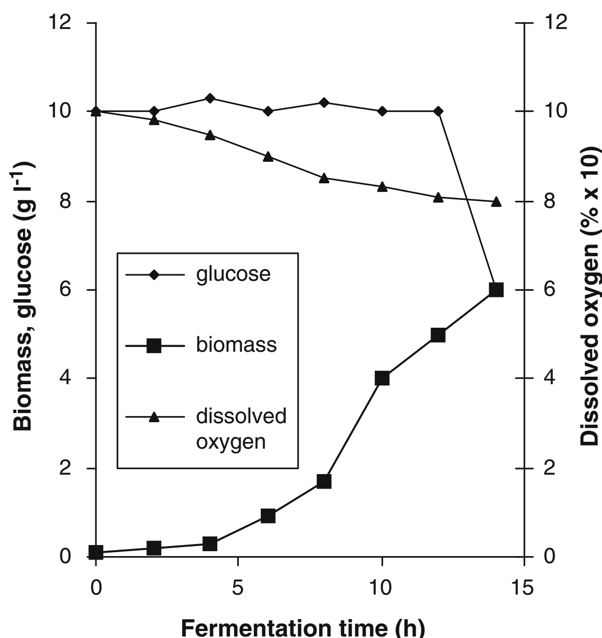

Fig. 1 Typical fed-batch fermentation of *S. cerevisiae* operated as glucostat at 10 g l^{-1} glucose

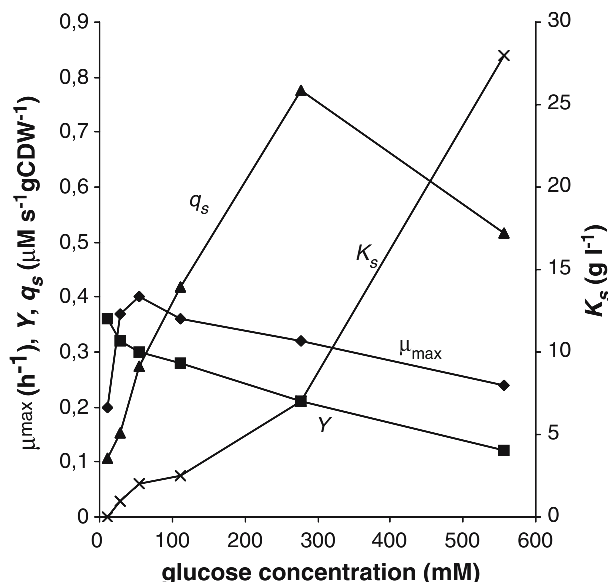

Fig. 2 Kinetic parameter values from glucostat cultures of *S. cerevisiae*

linear according to Fig. 2. However, the values obtained from glucostat cultures are much lower, compared to Y values from our batch fermentations and from batch cultures in the literature. Using an initial glucose concentration of 10 g l^{-1}, Kappeli [17, 18] reported the value of 0.5 for Y, which is equal to the value obtained in batch culture with an initial glucose concentration of 10 g l^{-1} in our strain, while the highest Y value obtained from glucostat data is 0.36 at 27.7 mM glucose (5 g l^{-1}). This could be explained by the elevated carbon dioxide production observed in the glucostat, which is found to increase with increasing glucose concentration levels. As expected, ethanol formation did set in when enhanced CO_2 production was observed (results not shown). Figure 3 gives information on the carbon distribution between carbon dioxide (maximum detected concentrations) and biomass (values corresponding to maximum detected concentrations of carbon dioxide) at various glucose concentration levels. The ratio carbon dioxide/biomass increases with increasing glucose levels because of the increased energy requirement under such conditions, which results in a low yield coefficient.

The effect of the maltose concentration level on growth kinetic parameters is shown in Fig. 4. Comparing Figs. 2 and 4, it is obvious that both sugars produced results of the same trend. However, the peak in the μ_{max} curve with glucose corresponds to 20 g l^{-1} glucose (or 111.1 mM) and the value equals 0.40 h^{-1}. After that point, a sharp drop was observed with the lowest μ_{max} value at 0.24 h^{-1} corresponding to 100 g l^{-1} glucose (555.5 mM). With maltose, the peak in the μ_{max} curve corresponds to 10 g l^{-1} maltose concentration level and the value is 0.35 h^{-1}. Beyond that point, a slight decrease in μ_{max} values was observed with the lowest value of 0.27 h^{-1} corresponding to 100 g l^{-1} maltose concentration level (or 277.5 mM). μ_{max} values of *S. cerevisiae* grown under stable maltose concentration levels appear to be higher than that observed with glucose at elevated sugar concentration levels. This is in contrast with the observations on batch cultures performed with various initial sugar concentrations by Barford and Hall [3], who reported that maltose-grown cultures had lower specific growth rates compared to the glucose-grown. With maltose, q_S increased up to the concentration level of 208.15 mM to drop at higher concentration levels. The yield coefficient Y on maltose appears to be slightly higher compared to the observed on glucose. In chemostat culture, Weusthuis et al. [50] found that the yield coefficient on maltose was significantly lower, by an almost 25%, compared to the one obtained from glucose. K_S values in the case of the maltose substrate remained at stable low levels at maltose concentrations above 138.7 mM and up to 277.5 mM (50–100 g l^{-1}).

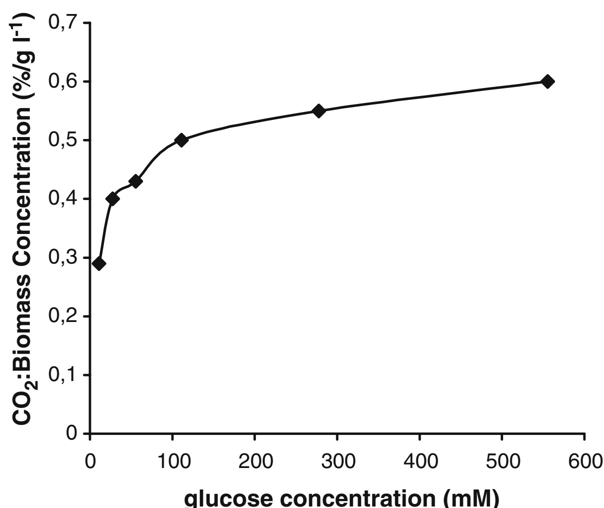

Fig. 3 The ratio of CO_2:biomass concentration at various glucose concentration levels in glucostat culture

Substrate inhibition effects in the maltose case are explained by the Crabtree effect as in the case of glucose. From the above description of the experimental data, it appears that in fed-batch culture operated at stable sugar concentration levels, substrate inhibition by maltose appears to be milder than the observed by glucose. The only explanation to this could be a higher level of glycolytic enzymes and a higher oxidative activity in maltose-grown than in glucose-grown cells, which results in milder catabolic repression. It is long known [33] that repression of the formation of respiratory enzymes in mitochondria of *S. cerevisiae* can be caused by formation of ''high-energy'' substances, e.g., ATP. ATP measurements in cells from samples taken at points corresponding to the maximum specific growth rates show that concentrations remain at rather low levels and within narrow limits (35–40 μg l^{-1}) at elevated maltose concentration levels (above 50 g l^{-1} and up to 100 g l^{-1}). The reason for this could be the higher amount of energy needed for maintenance of respiratory enzymes during growth in maltose which keeps the levels of ATP synthesis at lower levels.

In batch cultures performed with both sugars in the medium, maltose, as expected, was consumed following glucose depletion. Kinetic parameters obtained from batch experiments with media containing glucose and maltose together (10 g l^{-1} each, in MPYG Medium), maltose as the sole sugar source and glucose as the sole sugar source at an initial sugar concentration of 10 g l^{-1}, are plotted in Fig. 5. Comparing the values of μ_{max}, K_S and Y obtained from the medium containing both sugars and the medium with glucose only,

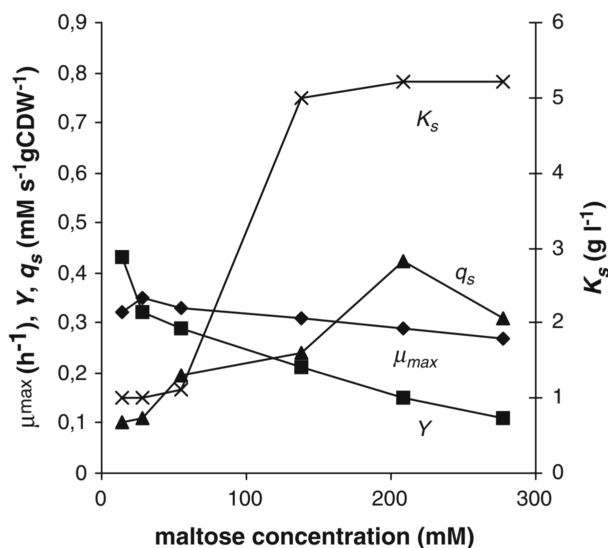

Fig. 4 Kinetic parameter values from fed-batch cultures of *S. cerevisiae* operated at constant maltose concentration levels

we observe that K_S is almost similar, while μ_{max} is lower in maltose-grown culture and the yield coefficient Y is higher in maltose-grown than in glucose-grown cultures. The higher Y may be explained by a milder repression in oxidative enzymes in maltose-grown cells. In batch culture, cells are experiencing the effects of a changing glucose concentration during the run; a situation that might lead in misinterpretation of results. In this case however, the higher yield coefficient on maltose than on glucose is observed in both

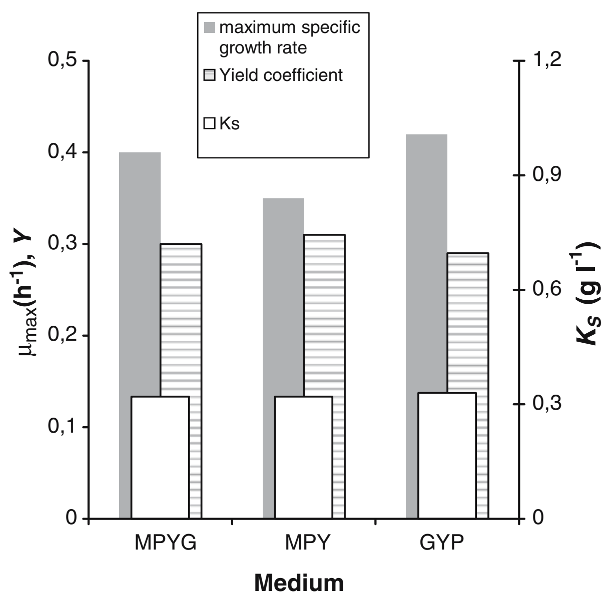

Fig. 5 μ_{max}, K_S and Y values obtained from batch fermentations with media containing glucose and maltose together or each sugar as a sole sugar source, at an initial concentration of 10 g l^{-1}

batch and fed-batch culture performed at stable maltose concentration levels.

Biochemical changes, control of glycolysis and the Crabtree effect in aerobic *S. cerevisiae* fermentations have been discussed in a large number of publications spanning many decades back. To our knowledge, there is no report on fed-batch culture kinetics, when this is operated as glucostat. It is not the aim of this work to discuss the biochemistry of the fermentation but to give a picture of the dynamics of the system under constant glucose or maltose concentration levels. Typical literature values for the kinetic parameters μ_{max}, K_S and Y are shown in Table 1. Literature information on K_S and Y is rather rare even for glucose substrates. Although direct comparisons of systems operated under fundamentally different conditions are quite impossible, we give the values for the purpose of reference. Obviously, kinetic parameter values may vary widely but it is not unusual that such values are adopted from literature sources and used in modeling works. Modeling of yeast growth has been the subject of a large number of publications in which a number of different rate expressions have been proposed. Some of these expressions are given in Table 2.

According to Figs. 2 and 4, at the sugar concentration level of 10 g l^{-1}, no substrate inhibition is likely to occur. Batch fermentations carried out as reference for fed-batch cultivation in this work, show that yeast growth follows Monod kinetics. The observed sugar inhibition effect in glucostat cultures was taken into account by using the model equation proposed by Jackson and Edwards [14] in their study on substrate inhibition kinetics in yeast. K_i is the inhibition coefficient expressed in g l^{-1}. This model was fitted to the experimental data from glucostat fermentations, having taken into account the amount of sugar added in the feeding process, for the sugar (glucose or maltose) concentration levels of 20, 50, 75 and 100 g l^{-1}, for selected K_i values. The runs operated until sugar depletion. Figures 6 and 7 present the kinetic model fitted to fed-batch cultures operated at stable concentrations of 100 g l^{-1} glucose and 75 g l^{-1} maltose, respectively. Open symbols correspond to the model-created values, to which trend lines are fitted. Close symbols and asterisk symbols correspond to experimental values. The K_i value in the glucose case (100 g l^{-1}) was 1,500 g l^{-1}. This was also the K_i value in the 75 g l^{-1} maltose case. In all sugar concentration levels, the model showed good fit to experimental data, as selectively shown in Figs. 6 and 7. The extent of inhibition is given by the demonstration, in Fig. 8, showing the inhibition coefficient K_i used to obtain the fit of the model with the experimental data. Table 3

Table 1 A selection of typical literature values for μ_{max}, K_S and Y in yeast fermentations

Microorganism	Culture	Sugar source	Kinetic parameters			Reference
			μ_{max} (h^{-1})	K_S ($g\ l^{-1}$)	Y ($g\ g^{-1}$)	
S. cerevisiae	Aerobic chemostat pH 5.0, 30°C	Glucose	0.45		0.17	Rieger et al. [36]
S. cerevisiae	Aerobic chemostat pH 5.0, 30°C	Glucose	0.49		0.16	Postma et al. [34]
S. cerevisiae	Aerobic batch	Glucose	0.45			Barford and Hall [3]
		Sucrose	0.40			
		Maltose	0.28			
		Galactose	0.23			
	Aerobic chemostat pH 5.0, 30°C	Glucose	0.45			
		Galactose	0.23			
S. cerevisiae	Aerobic batch pH 4.6, 30°C	Glucose	0.41			Parada and Acevedo [29]
S. cerevisiae	Aerobic batch	Glucose	0.54	0.034		Jain [15]
	Anaerobic batch pH 5.8, 30°C		0.50	0.187		
S. cerevisiae	Aerobic chemostat pH 6.0, 30°C	Glucose	0.65	0.108		Leuenberg [24]
S. cerevisiae	Aerobic batch	Glucose	0.44			Auling et al. [2]
	Anaerobic batch pH 5.0, 30°C		0.29			
S. cerevisiae	Aerobic chemostat pH 4.0, 30°C	Glucose	0.49	0.146		O'Neil and Lyberatos [28]
S. cerevisiae	Anaerobic chemostat pH 5.0, 30°C	Glucose	0.31	0.099		Verduyn et al. [46]
Saccharomyces uvarum	Aerobic chemostat pH 5.5, 30°C	Glucose	0.26			Petrik et al. [32]
Saccharomyces calsbergensis	Aerobic batch	Glucose	0.39		0.52	Toda et al. [41]
	Aerobic chemostat pH 4.6, 30°C		0.55	0.021	0.45	

Table 2 A selection of kinetic models used for yeasts

Microorganism	Kinetic expressions	Reference
S. cerevisiae	$\mu = \mu_{max}\frac{S}{S+K_S}$	Monod [26]
S. cerevisiae	$\mu = \mu_{max}\frac{S}{S+K_S}^{1-\exp(-t/T1)}$	Bergter and Knorre [5]
S. cerevisiae	$\mu = \mu_{max}\frac{S^n}{S^n+K_S}$	Moser [27]
S. cerevisiae	$\mu = \mu_{max}\frac{S}{S+K_S}\frac{1}{1+bC_L}$	Peringer et al. [31]
S. cerevisiae	$\mu = \mu_{max}\frac{(1-P)^n}{P_m}$	Levespiel [25]
Candida utilis	$\mu = \mu_{max}\frac{S}{K_S+S+S^2/K_i}$	Jackson and Edwards [14]

summarizes the kinetic parameter values obtained in fed-batch cultures at various sugar concentration levels. Interestingly, the values obtained for K_i do not vary with the sugar source. Although in maltose, μ_{max} was found to be slightly lower than the one obtained in glucose, the inhibition coefficient of the sugar was the same for both sugar sources.

Fed-batch culture is the mode of operation of choice in industrial baker's yeast fermentation. We operated the fed-batch as glucostat to estimate important kinetic parameters in a process mostly described in the literature as batch or chemostat fermentation. Comparing the kinetics of growth and the inhibition by the substrate, in cultures grown on glucose, which is the preferential sugar source for *S. cerevisiae*, and maltose, the most common sugar source in industrial fermentation media for baker's yeast production, we observed a milder inhibition effect by the substrate in maltose-grown cells and a higher yield coefficient. The inhibition coefficient K_i appears to be in the same levels for both substrates at elevated concentration levels.

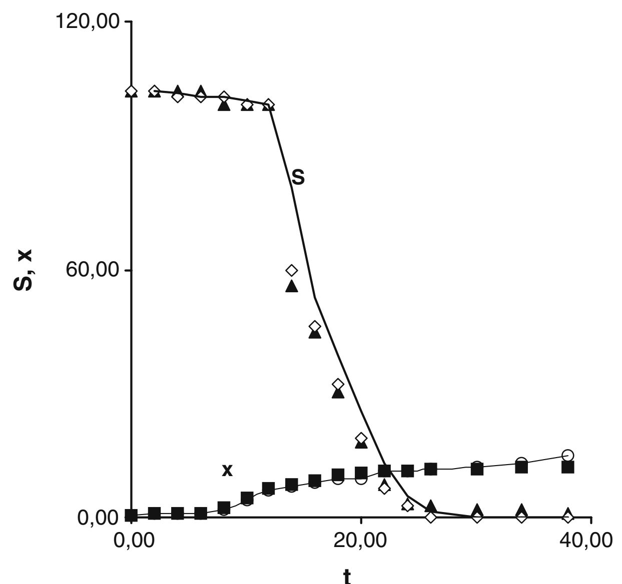

Fig. 6 The kinetic model fitted to glucostat culture operated at 100 g l^{-1} glucose concentration. *Open symbols* correspond to model-created values. *Close symbols* and *asterisk symbols* correspond to experimental values. x is the biomass concentration (g l^{-1}), S is the concentration of glucose (g l^{-1}) and t is the fermentation time (h)

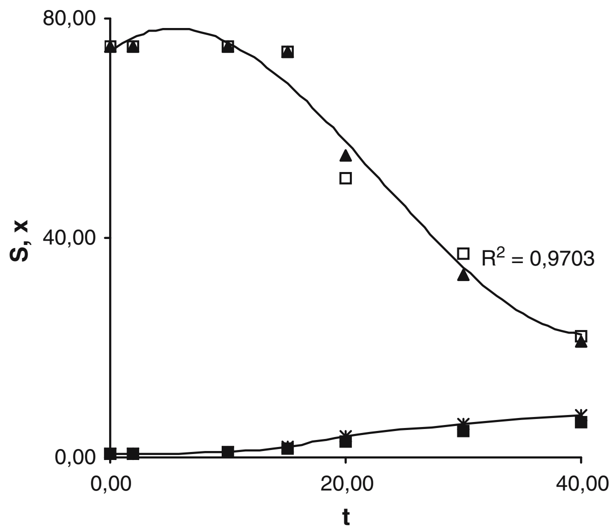

Fig. 7 The kinetic model fitted to fed-batch culture operated at 75 g l^{-1} maltose concentration. *Open symbols* correspond to model-created values. *Close symbols* and *asterisk symbols* correspond to experimental values. x is the biomass concentration (g l^{-1}), S is the concentration of glucose (g l^{-1}) and t is the fermentation time (h)

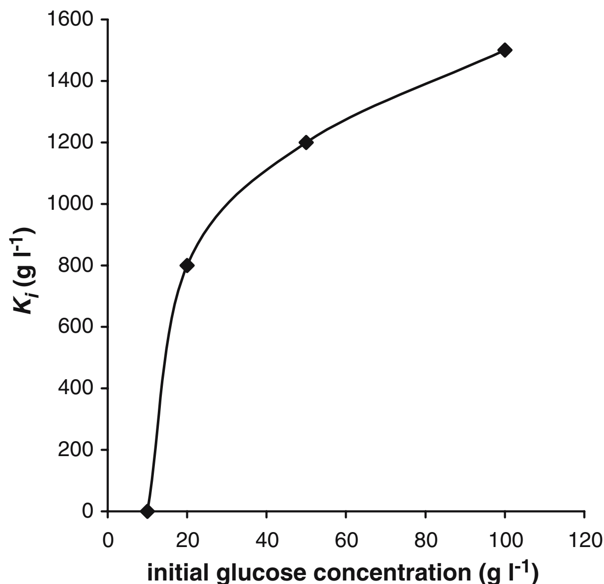

Fig. 8 The relationship between glucose concentration level and inhibition coefficient K_i in glucostat experiments

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Table 3 A summary of the kinetic parameters' values obtained in fed-batch cultures operated at various sugar concentration levels

Sugar concentration (g l^{-1})	Glucose			Maltose		
	μ_{max} (h^{-1})	K_S (g l^{-1})	K_i (g l^{-1})	μ_{max} (h^{-1})	K_S (g l^{-1})	K_i (g l^{-1})
10	0.37	0.80	0.00	0.35	0.8	0.00
20	0.40	1.51	0.80	0.33	0.8	0.75
50	0.36	6.50	1.20	0.31	5.0	1.20
75	0.32	1.20	1.50	0.29	6.0	1.50
100	0.24	28.00	1.50	0.27	5.3	1.50

proved maltose affinity and hypersensitivity. Appl Environ Microbiol 70:1956–1963
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