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EFFECT OF METHANOL CONCENTRATION ON THE RECOMBINANT *PICHIA PASTORIS* MUT^S FERMENTATION

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Abstract

Methanol is the sole carbon and energy source as well as inducer of heterologue protein production in recombinant *Pichia pastoris* fermentations. The role of methanol on *P. pastoris* GS115 Mut^S HSA model strain was thoroughly studied in a series of fed-batch cultivations at different constant alcohol levels in bench-top and pilot-scale bioreactors. The results showed that whereas specific growth rate does not show correlation with methanol concentration, specific product formation rate is strongly dependent on substrate level. The fact that the specific growth rate did not decrease with increasing methanol concentrations can be explained by the diminished toxic metabolic compound formation compared to *P. pastoris* Mut$^+$ cells. While metabolic inhibition was not characteristic in the range of methanol concentration 0.45–8.85 g/L, the product formation was mainly controlled by the initiation of transcription and was favoured by low methanol concentrations. As a result of the relatively high value of maintenance coefficient (0.026 1/h), all fermentations were performed in energy limit which explains that the specific product formation rate was not correlated to the specific growth rate. The highest value of volumetric productivity (0.0187 mg HSA/(L*h)) was measured at 0.45 g/L methanol concentration. The values of specific metabolic quotients as functions of methanol concentration in scaled-up fermentations coincided with the results in bench top reactors when adequate mixing was applied.

Keywords: Pichia pastoris, fermentation, methanol concentration, specific product formation rate.

1. Introduction

The expression system based on the yeast *Pichia pastoris* is a widely used and effective tool for recombinant protein production [1, 2]. As a result of the intensive research in the last two decades, the process is at the stage of industrial application [3, 4]. For the successful scale up to production scale, right choice of process parameters and tight control are necessary. The optimal production technique requires proper understanding of the physiology and fermentation of this methanogen yeast [5]. The most crucial process parameter in determining final product concentration and volumetric productivity is the concentration of methanol.

P. pastoris is capable to use methanol as sole carbon and energy source. The methanol is oxidized by the alcohol-oxidases of this yeast. The alcohol-oxidases are produced by the two AOX genes of *Pichia*: AOX1 and AOX2 [6, 7]. Whereas the protein coding regions of the functional genes and the protein products are 92% and

97% homologous, respectively, the promoter regions are distinct: AOX1 produces approximately 90% of the alcohol oxidase in the methanol induced state of the cells. The strong AOX1 promoter is used for constructions of recombinant protein expressing casettes which are integrated into the genome of the yeast, resulting strains in which the recombinant protein production is under the control of the methanol concentration in the media [8, 9]. Thus, methanol serves as the inducer of product formation as well as energy and carbon source.

Two major variations of recombinant *P. pastoris* are used for production purposes: the Mut^+ (Methanol utilization type +) has intact AOX1 and AOX2 genes whereas the Mut^S (Methanol utilization type Slow) possesses only intact AOX2 gene [10]. The expression of the heterologue gene is controlled by the AOX1 promoter in both cases. Even though the Mut^S variant has a diminished maximal growth rate compared to Mut^+, the final concentration of the functional product protein is occasionally higher in the case of Mut^S fermentations as a result of the proper post-translational modifications [11, 12]. Also, in case of too fast recombinant protein formation, the product may not be fully secreted and the excess protein remains in the cytosol as protein aggregates [13].

The growth and product formation kinetics of the Mut^+ type are well characterized, whereas the Mut^S is less known [14, 15]. In case of the Mut^S, the optimum methanol concentration for product expression may differ from optimum for the alcohol-oxidase formation as a result of the different control promoters, e.g. pAOX1 and pAOX2. Since the available energy in the cells largely influences the translation and secretion of the recombinant protein, the maximal specific product formation rate intricately depends on the concentration of methanol, which acts as energy and carbon source as well as inducer of the expression. Furthermore, the volumetric productivity of the recombinant protein is expected to show more complex behaviour as it is interfered by both specific product formation and growth rates. A reliable correlation describing the specific product formation rate as the function of the specific growth rate was not established in case of Mut^S cells and even complex experimentally supported kinetic models were insufficient to predict product formation without involving the direct dependence of specific product formation rate on methanol concentration [16]. Accepting the key role of methanol in product formation, our purpose was to investigate the effect of methanol concentration on the kinetic parameters of a human serum albumin producing model strain of *P. pastoris* Mut^S [17, 18].

2. Material and Methods

2.1. Strain

Pichia pastoris GS115 Mut^S with a single copy of human serum albumin V (HSA) gene under the control of AOX1 promoter purchased from Invitrogen Co. (USA) was used. It was maintained on YEPD agar slants at $+4\ ^\circ C$ (20 g/L glucose,

20 g/L bacteriological peptone (Oxoid, USA), 10 g/L bacto yeast extract (Difco Laboratories, Becton, Dickinson & Co., USA), 20 g/L agar-agar). All chemicals were purchased from Reanal (Hungary) unless indicated differently.

2.2. Cultivation

Inoculum was prepared in a gyrotory shaker (type G25, New Brunswick, USA) at 29 °C, 300 rpm. Composition of inoculum media was the following: 10 g/L glycerol, 13.4 g/L Yeast Nitrogen Base with amino acids (Sigma-Aldrich, USA), 20 g/L peptone, 10 g/L yeast extract, 0.4 mg/L biotin (Sigma-Aldrich, USA) in 0.1 M potassium-phosphate buffer (pH 6.0). 120 and 1000 mL of the 24-hour-old shake-flask cultures were used to inoculate the bench top and pilot scaled fermentors, respectively.

Cultivations to evaluate influence of methanol concentration were carried out in 1200 mL working volume bench top Biostat M bioreactor (B. Braun Biotech, Germany) at controlled pH and temperature. 1000 rpm agitation speed and 1.6 L/min aeration rate were applied. Pilot scaled fermentations were performed in B. Braun Biostat U system applying U20 type stirred tank reactor or 883 822/4 type air-lift reactor. In both cases the volume of fermentation broth and the aeration were 19 litres and 2 Nm^3/h, respectively. The agitation in the U20 reactor was 500 rpm performed by three flat blade (Rushton) turbine impellers. All fermentation parameters except substrate concentration and dry cell weight were registered and controlled by MFCS II data acquisition software of B. Braun Biotech.

The cultivation medium composition was as follows: 40 g/L glycerol, 18.2 g/L K_2SO_4, 14.9 g/L $MgSO_4$, 4.13 g/L KOH, 0.93 g/L $CaSO_4 \cdot 7H_2O$, 26.7 ml/L cc. phosphoric-acid and 4.35 ml/L of PTM_1 trace element solution (concentrations are given as final data including inoculum volume). The composition of the trace element solution was [19]: 65.00 g/L $FeSO_4 \cdot 7H_2O$, 20.00 g/L $ZnCl_2 \cdot 4H_2O$, 6.00 g/L $CuSO_4 \cdot 5H_2O$, 1.50 g/L $MnSO_4 \cdot H_2O$, 0.25 g/L $CoCl_2 \cdot 6H_2O$, 0.10 g/L $NaMoO_4 \cdot 2H_2O$, 0.10 g/L biotin and 0.04 g/L NaI.

The nutrient feed in the production phase consisted of analytical grade pure methanol with 12 ml/L PTM_1 solution. The methanol concentration of the fermentation broth was controlled at constant level as described later.

The fermentation procedure started with a batch phase on glycerol at pH 5.0 and 29 °C. The depletion of glycerol from the system was indicated by the rise of the dissolved oxygen level at the end of the batch phase. When the dissolved oxygen level increased, the pH and temperature of the fermentation broth were adjusted to 5.7 and 20 °C, respectively, along linear gradient in 30 minutes. The pH was controlled by 25% NH_4OH and 25% H_2SO_4 solutions. The glycerol batch phase was followed by a 20-hour-long adaptation period on methanol with a constant 0.66 ml/(L*h) methanol feed rate. This period of low feed rate served as inducer of the alcohol-oxidase system of the yeast as well as to eliminate metabolically the acetate and ethanol, which is reported to accumulate occasionally in the fermen-

tation broth during the glycerol phase and inhibit growth on methanol [20]. The subsequent methanol feed phase, where the substrate concentration was maintained at the desired level, lasted for 50–55 hours.

2.3. Control of Methanol Concentration During the Production Phase

Methanol concentration was kept at the desired level by a closed loop control system similar to that described by Katakura [21]. The method is based on the observation that no other organic vapour is present in the media during the fermentation process beside methanol and this way the measurement of total organic vapour concentration can be applied to follow methanol level in the fermentation broth. A steady airflow was led through a silicon tube submerged into the fermentation broth. Reaching a steady state, the methanol tension in the air flow becomes proportional to the dissolved methanol concentration of the fermentation broth. The airflow was directed onto a Figaro TGS 822 organic vapour sensor (Figaro Engineering Inc., Japan). The SnO_2 particles of the sensor change their electric resistance by encountering with methanol molecules which results in an electrical signal. This signal was amplified and served as a measure of the methanol level in the fermentation broth. An on/off control unit turned on an infusion driver (Kutesz, Hungary) when the methanol concentration fell below the desired level and turned it off if the alcohol concentration reached the set-point again. The system was calibrated prior to each fermentation.

2.4. Analytical Methods

Biomass concentration was determined from the optical density of the fermentation broth at 600 nm (Pharmacia LKB-Ultrospec Plus, Pharmacia Co., USA). Biomass is expressed as dry cell mass, which was calibrated to the optical density ($DCW = 0.45 \cdot \mathrm{OD}_{600}$).

Glycerol concentration was determined by off line high-performance liquid chromatography analysis (LKB Bromma, Aminex®HPX-87H Ion Exclusion Column 300 nm $\times$ 7.8 mm, Bio-rad, USA), using the following conditions: sample volume – 40 μL; mobile phase – 0.01 N H_2SO_4; flow rate – 0.5 mL/min; column temperature – 65 °C. Methanol concentration was also measured off line by gas chromatography (Laboratorni Pristroje Praha, Chrom4 GC system, Czech R., column – 0.2% Carbowax 1500 on 80/100 Carbopack C, 6', Supelco, USA; sample volume – 3 μL; mobile phase – nitrogen; column temperature – 70 °C, detector – FID).

The HSA concentration was determined from the cell free supernatant of fermentation samples by gradient SDS gel electrophoresis (Pharmacia Phast System, Phast Gel Gradient 8–25 gel [Amersham-Pharmacia, Sweden], crystallized HSA

V standard [Sigma, USA], silver staining, evaluated by Kodak Digital Science 1D Image Analysis Software).

3. Results

The purpose of this work was to find a functional relationship between the methanol concentration and various metabolic quotients like the specific growth rate (q_x), specific substrate consumption rate (q_{MeOH}) and specific product formation rate (q_P) during the production phase of a *P. pastoris* Mut^S fermentation. We assumed that the controlled methanol concentration in the production phase resulted in steady state which was characterized by constant values of the specific rates at the given substrate level. The calculations of the specific rates were the followings:

$$\bar{x} = \frac{1}{t_z - t_1} \cdot \sum_{n=1}^{z} \left(\frac{x_{n-1} V_{n-1} + x_n V_n}{2} \cdot (t_n - t_{n-1}) \right), \tag{1}$$

$$q_x = \frac{1}{\bar{x}} \cdot \frac{x_z - x_1}{t_z - t_1}, \tag{2}$$

$$q_{\mathrm{MeOH}} = \frac{1}{\bar{x}} \cdot \frac{m_{\mathrm{MeOH}}}{t_z - t_1}, \tag{3}$$

$$q_P = \frac{1}{\bar{x}} \cdot \frac{c_{\mathrm{HSA},z} V_z - c_{\mathrm{HSA},1} V_1}{t_z - t_1}. \tag{4}$$

The total consumed methanol weight (m_{MeOH}) was considered to be the total weight of methanol fed into the fermentor during the production period, corrected by the amount of methanol evaporated with the aeration. The amount of evaporated substrate was calculated as described earlier [22].

To evaluate the effect of substrate concentration, we performed fermentations in bench top reactors keeping seven different methanol levels between 0.45 and 8.85 g/L during the production phase (*Table 1*). The success of the above explained on/off control of methanol concentration is shown in *Fig. 1*.

P. pastoris GS115 is known to produce minor amounts of extracellular proteases if nitrogen starvation occurs [23]. Our calculations showed that the fermentation broth contained surplus nitrogen in all cases which resulted in that no significant HSA degradation was observed in SDS PAGE.

Whereas the specific growth rate (q_x) did not show strong dependence on methanol concentration, the specific product formation rate (q_P) had two maxima in the function of methanol: 0.00056 and 0.00035 g HSA/g CDW/h at 0.45 and 3.58 g/L methanol, respectively. Since q_x was not affected by the methanol concentration as drastically as q_P, the volumetric productivity showed similar characteristics in the function of methanol concentration as q_P and had its maximum of 0.0187 g HSA/L/h at 0.45 g/L methanol.

Table 1. Summary of results measured in the methanol-stat period of the fermentations. * 19 litres stirred tank reactor; ** 19 litre air-lift reactor

Methanol g/l	Final HSA mg	Final Cell MASS g CDW/L	$Y_{P/\mathrm{MeOH}}$ g/g MeOH	$Y_{X/\mathrm{MeOH}}$ g/g MeOH	q_{MeOH} g MeOH/(g CDW*h)	q_P g HSA/(g CDW*h)	q_X 1/h	J_P g HSA/(L*h)	J_X g CDW/(L*h)
0.45	1182	36.27	0.0161	0.153	0.0350	0.00056	0.0054	0.0187	0.262
1.45	625	42.08	0.0080	0.295	0.0288	0.00023	0.0065	0.0094	0.265
2.70	389	40.73	0.0021	0.113	0.0391	0.00008	0.0055	0.0030	0.177
3.58	664	44.28	0.0143	0.176	0.0243	0.00035	0.0043	0.0114	0.185
4.67	523	37.57	0.0068	0.244	0.0276	0.00016	0.0070	0.0055	0.219
6.04	465	40.55	0.0066	0.199	0.0285	0.00016	0.0054	0.0058	0.201
8.85	436	33.49	0.0076	0.323	0.0271	0.00021	0.0088	0.0046	0.209
*1.37	1352	124.4	0.0074	0.260	0.0296	0.00036	0.0073	0.0176	0.664
**0.50	2056	45.17	0.0019	0.224	0.0185	0.00029	0.0079	0.0122	0.270

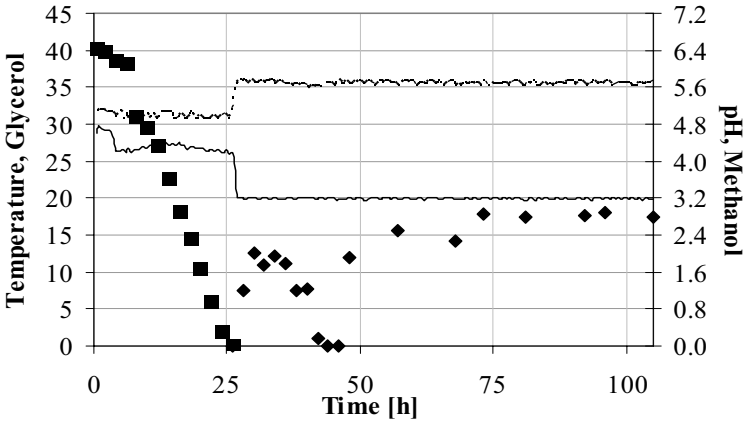

Fig. 1. Example for recombinant *P. pastoris* GS115 Mut^S fermentation with direct methanol concentration control in the 65th–105th hour range. ■ Temperature [°C]; — pH; glycerol concentration [g/L]; ♦ methanol concentration [g/L]

In order to evaluate if the results measured in bench top reactors can be generalized, pilot scale fermentations were conducted. Our purpose was to examine the possible influence of scale up on the methanol-dependence of specific rates in a 19 litres stirred tank reactor, which had similar geometric ratios as the stirred bench top reactor. Furthermore, 19 litres air-lift fermentation was performed to see if our results can be applied directly to other fermentor types. The results of the experimental runs show that the specific rates in the 19 litres stirred tank reactor coincided with the data measured in bench top fermentations, whereas the air-lift reactor showed inferior performance (*Table 1*).

4. Discussion

4.1. Evaluation of Measured Kinetic Parameters

By physiological considerations, the role of methanol on recombinant *P. pastoris* Mut^S can be summarized as follows. Specific growth rate is affected by methanol by the induction of transcription on AOX2 gene. AOX promoters are fully activated at substrate limited conditions [5]. On the other hand methanol, as the substrate of AOX enzyme, determines the rate of substrate oxidation. The oxidation of methanol is considered to be the rate limiting step of the whole oxidation process toward carbon-dioxide and the assimilating routes, as well. The reaction is characterized by a complex kinetic scheme of four consecutive steps even among single-turnover conditions [24]. The rate of methanol conversion determines the available energy and carbon for maintenance, cell growth, product formation and

product transport into the media. Although the oxidation of methanol takes place in membrane bordered cell organelle, called peroxysome, the toxic products of alcohol oxidation e.g. hydrogen-peroxide and formaldehyde have inhibitory effects directly on the AOX enzyme as well as demolish cell structures in general [25, 26]. As a result of these induction/inhibition effects of methanol, q_x can be characterized by uncompetitive inhibition kinetics in the function of substrate for the Mut^+ type [27]. The ideal alcohol concentration for cell growth is 3.65 g/L in this case. While the intact AOX1 locus supplies sufficient amount of alcohol-oxidase at higher substrate concentration range in Mut^+ cells, relative enzyme shortage characterizes the Mut^S variant. For this reason, the effect of methanol on induction is likely to have a more definite influence on q_x in Mut^S cells than in Mut^+. Furthermore, the slow transformation of methanol decreases the accumulation of oxidative metabolic products, diminishing the inhibitory effects in Mut^S. For the above reasons the q_x of *P. pastoris* Mut^S can not be characterized by a single peak uncompetitive inhibition kinetic model (*Fig. 2*). The q_{MeOH}, which is the linear function of q_x in Mut^+ cells among substrate limiting conditions [28], is rather independent both on q_x and the methanol concentration in Mut^S cells (*Fig. 3*). This phenomenon can be explained by that only a small portion of the substrate uptake is used for growth and product formation, whereas the majority of the energy source is required for maintenance. The observed maximum for q_{MeOH} was measured at 0.0055 1/h q_x value.

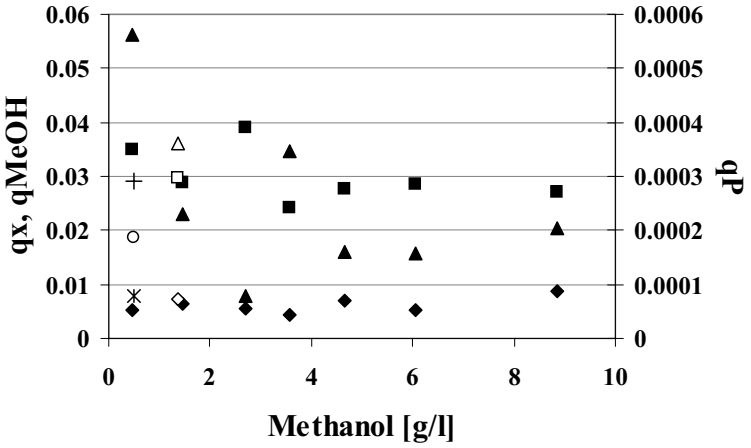

Fig. 2. Specific kinetic rates in the function of methanol. ♦ q_x [1/h]; ◊ q_{MeOH} [g MeOH/g CDW/h]; ▲ q_P [g HSA/g CDW/h]; ■ bench top reactor; □ 19 litres stirred tank reactor; ✳ q_x [1/h] in 19 litres air-lift reactor; ○ q_{MeOH} [g MeOH/g CDW/h] in 19 litres air-lift reactor; + q_P [g HSA/g CDW/h] in 19 litres air-lift reactor

The specific product formation rate is mainly influenced by methanol through the control of AOX1 promoter, which is responsible for the recombinant product formation. It is well established that AOX promoters are maximally induced at low

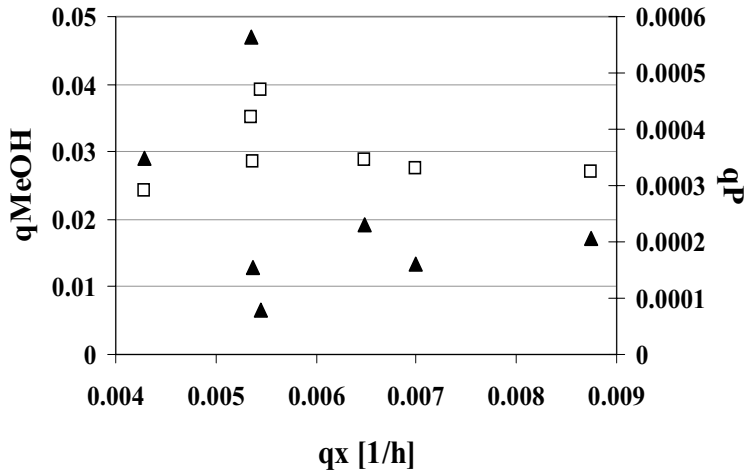

Fig. 3. Specific methanol consumption and product formation rate in the function of specific growth rate for bench top reactors. ▲ q_{MeOH} for bench top reactors [g MeOH/g CDW/h]; □ q_P for bench top reactors [g HSA/g CDW/h]

methanol concentrations, which denote limiting conditions for cell growth. The Mut^+ type cells produce sufficient amount of enzyme to satisfy the energy and carbon demand of the product formation even at higher substrate concentrations as a result of the active AOX1 site. The optimum for the substrate uptake, cell growth and energy metabolism is at these higher methanol concentrations. On the other hand, the product formation is limited by the initiation of product formation and the available energy and carbon source. For this reason, the maximum of q_P is between the optimum for growth (3.65 g/L) and intracellular product formation (0.34 g/L), at 2.1 g/L methanol for Mut^+ cells [29]. Again, as the result of low enzyme content, the energetic and induction optima separates in Mut^S cells: even though a local maximum of q_P was observed at 3.6 g/L methanol, the maximum q_P is clearly determined by the promoter induction efficiency and shows the highest value at 0.45 g/L. Thus the specific product formation rate in *P. pastoris* Mut^S cells show similar exponential decrease with increasing methanol concentrations as the alcohol-oxidase based expression system of *Hansenula polymorpha* [30]. It is important to emphasize that this effect is mainly denoted to the decreasing induction of AOX1 promoter towards higher methanol concentrations and not to the diminished inhibition by oxidized metabolic products. An analogous discrepancy stands for q_P versus q_x between Mut^+ and Mut^S *P. pastoris*: in Mut^+ cells q_P showed a single maximum at 0.015 1/h [29] while q_P apparently does not have direct dependence on q_x in the Mut^S variant.

Overall productivities for cell mass and HSA show similar dependence on

methanol concentration as the specific rates. The highest productivity value for the recombinant product (0.0187 g HSA/g CDW/h) was observed at 0.45 g/L substrate concentration.

The reciprocal of overall yield for cell mass shows linear dependence on the reciprocal of q_x according to *Eq.* (5):

$$\frac{1}{Y_{x/\mathrm{MeOH}}} = \frac{1}{Y^{T}_{X/\mathrm{MeOH}}} + \frac{m}{q_x} \tag{5}$$

The value of maintenance coefficient (m) calculated from the experimental data is 0.026 1/h (*Fig. 4*), which perfectly coincides with the value published by Pais [31]. Interestingly, the product yield has linear correlation with q_x (*Fig. 5*), which supports the observation that the product formation is performed among energy limit.

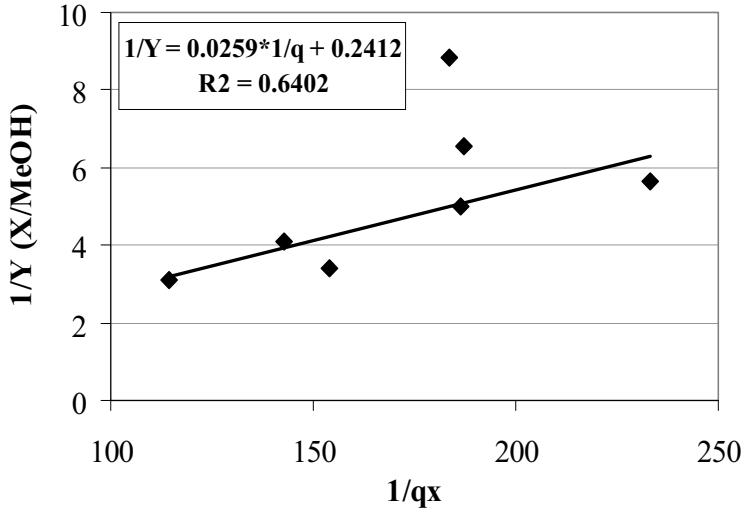

Fig. 4. Reciprocal of the cell yield in the function of reciprocal of the specific growth rate for bench top reactors

4.2. Comparison of Investigations with 1.2 and 19 Litres Medium Volumes

The experimental values of specific rates are represented in *Fig. 2* for the 19 litre fermentations in stirred tank and air-lift bioreactors. While the values of specific rates in the stirred tank reactor coincide with the results measured in bench top reactors, the fermentation performed in air-lift reactor has shown different behaviour.

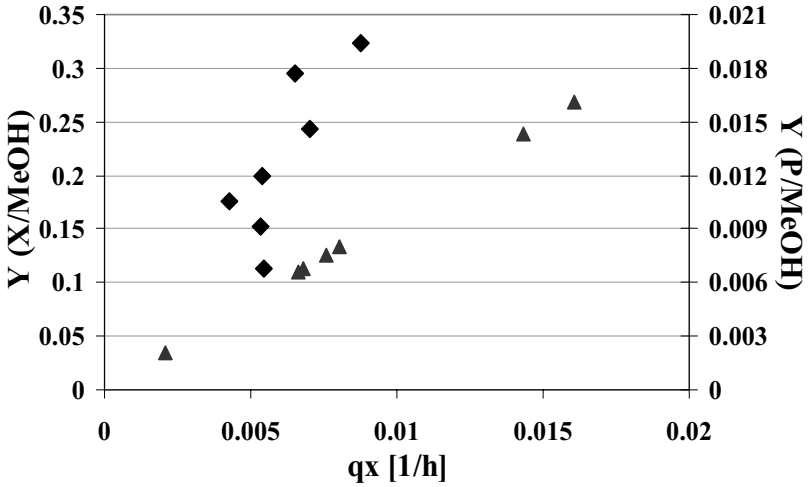

Fig. 5. Yields in the function of specific growth rate. ♦ $Y_{X/\mathrm{MeOH}}$ for bench top reactors [g DCW/g MeOH]; ■ $Y_{P/\mathrm{MeOH}}$ for bench top reactors [g HSA/g MeOH]

A putative reason for the inferior results in the air-lift reactor can be explained by the extreme sensitivity of Mut^S *P. pastoris* to the improper agitation and uneven aeration. In spite of this limitation, the values of specific rates measured in bench top reactors as a function of methanol seem to be valid for carefully stirred tank reactors in larger scale.

4.3. Statistical Model of Pichia pastoris Mut^S Fermentation

Every experimental evidence of *P. pastoris* Mut^S fermentations show strong substrate concentration dependence during the production period. The prerequisite of high and reliable productivity is the constant methanol concentration in the fermentation broth. The control of methanol level is usually based on three distinct approaches: (1) the direct measurement of methanol; (2) the control of substrate addition based on changes in the dissolved oxygen level or (3) on a preliminarily determined methanol feed profile, which is set up according to kinetic considerations. In cases of the direct measurement of methanol and the DO-based control system, a general problem is the fluctuation of methanol concentration in the fermentation broth, however this problem can be overcome by PID control in case of direct methanol measurement [32]. In spite of that the specific production rate strongly depends on the substrate level in the fermentation broth in *P. pastoris* Mut^S cells, as our results showed, the design of oxygen controlled *Pichia* cultivations rarely take into account the strong influence of fluctuating methanol concentration on produc-

tion rates. Also, even the most complex models based on theoretical assumptions to design subtle substrate feed profiles do not include the unusual behaviour of specific product formation rate and specific substrate consumption rate, consequently the practical applicability of these models are highly questionable [33]. In order to avoid the simplification of q_{MeOH} and q_P to linear dependence on q_x, we set up fed batch and continuous fermentation models by characterizing all specific rates barely by polynomials fitted to our experimental data. This way the specific rates are solely determined by the methanol concentration of the fermentation broth and are independent from each-other. The experimental design limits the application of these models to the 0.45–8.85 g/L methanol concentration range and thus are only useful to design substrate-stat cultivations with preliminarily determined methanol feed profile or to model fermentations with on-line methanol control in the characterised concentration range for productivity calculations. An example for the latter application with the calculated and measured values of a fed-batch fermentation is shown in *Fig. 6*.

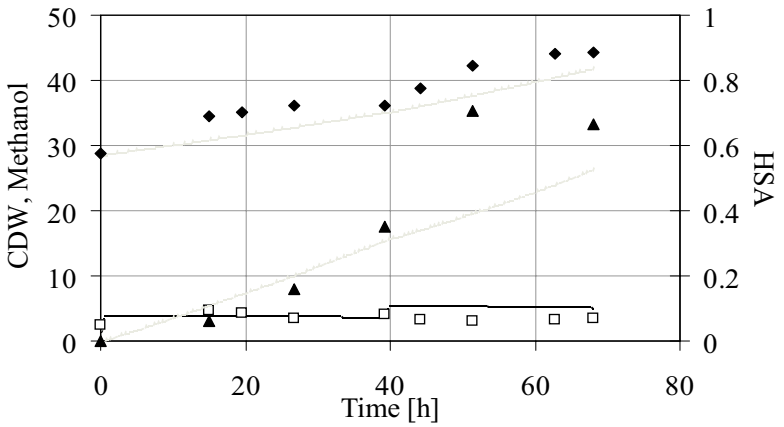

Fig. 6. Predicted and measured values of statistical model run of a fed-batch *P. pastoris* GS115 Mut^S HSA fermentation. - - - predicted cell dry weight [g/L]; ♦ measured cell dry weight [g/L]; — predicted methanol concentration [g/L]; ▲ measured methanol concentration [g/L]; ··· predicted human serum albumin concentration [g/L]; □ measured human serum albumin concentration [g/L]

5. Conclusions

The specific growth rate of recombinant *P. pastoris* Mut^S cells show less variation in the function of methanol concentration than Mut$^+$ cells possibly as a result of

diminished intracellular accumulation of toxic oxidative metabolic products and the relatively high energy need for maintenance. As a result of limitation in available energy, the specific product formation rate of the recombinant model protein HSA was mainly determined by the initiation of transcription and was favoured by low methanol concentration (0.45 g/L). Unlike in case of Mut^+ cells, the growth rate did not have strong influence on the volumetric productivity, which changed parallel with the specific product formation rate and had its maximum at substrate limiting conditions. The scale up of the fermentation did not alter the values of the specific rates in the function of methanol if proper mixing was maintained. The experimentally determined methanol concentration dependent specific rate values can be directly applied for productivity predictions of *P. pastoris* fermentations with on-line methanol control or to design methanol feed profile for substrate-stat fermentations.

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Abbreviations

HSA	human serum albumin
$\bar{x}$	average cell concentration in fermentation broth [g CDW/L]
t_n	fermentation time at constant methanol concentration at sample 'n' [h]
x_n	cell concentration at sample 'n' [g CDW/L]
V_n	volume of fermentation broth at sample 'n' [L]
q_x	specific growth rate [1/h]
q_{MeOH}	specific methanol consumption rate [g MeOH/g CDW/h]
m_{MeOH}	sum of methanol weight added to the fermentation broth [g]
q_P	specific product formation rate [g product/g CDW/h]
$c_{\mathrm{HSA},n}$	HSA concentration in fermentation broth at sample 'n' [g HSA/L]
$Y_{X/\mathrm{MeOH}}$	cell yield on methanol [g CDW/g methanol]
m	maintenance coefficient [1/h]
CDW	cell dry weight [g]
$Y_{P/\mathrm{MeOH}}$	product yield on methanol [g product/g methanol]
J_P	volumetric productivity for product [g product/h/L fermentation broth]
J_x	volumetric productivity for DCW [g DCW/h/L fermentation broth]

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