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Oxygen enriched air supply in *Escherichia coli* processes: production of biomass and recombinant human growth hormone

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Abstract

In order to investigate the impact of high oxygen and carbon dioxide concentrations, *Escherichia coli* was grown in batch cultivations where the air supply was enriched with either oxygen or carbon dioxide. The effect of elevated concentrations of oxygen and carbon dioxide on stochiometric and kinetic constants was studied this way. The maximum growth rate was significantly reduced, the production of acetic acid and the biomass yield coefficient on glucose increased in cultures with carbon dioxide enriched air, compared to reference cultivations and cultivations with oxygen enriched air. The application of oxygen enriched air was studied in high cell density cultivations of *Escherichia coli*. Two production processes were chosen to investigate the impact of oxygen enrichment. Biomass concentration, specific growth rate, yield coefficient, respiration, mixed acid fermentation products and the product yield and quality for the recombinant product were investigated. First, a process for the production of biomass was investigated. Exponential growth could proceed for a longer time and higher growth rates could be maintained with oxygen enriched air supply. However, a higher specific oxygen consumption rate per glucose was measured after the start of the oxygen enrichment, indicating higher maintenance and consequently the growth rate and yield coefficient decreased drastically in the end of the process. Second, a process for the production of recombinant human growth hormone (rhGH) was investigated. Although the glucose feed rate and all medium components were doubled, the amount of produced biomass could only be increased by 77% when oxygen enriched air (40% oxygen) supply was applied. This was due to a decreased yield coefficient of biomass per glucose. The total amount of produced product was decreased by almost 50% compared to the control, although less proteolytically degraded variants were produced. © 2002 Elsevier Science Inc. All rights reserved.

1. Introduction

The use of fed-batch cultivation technique for the production of recombinant proteins in *E. coli* has been extensively studied in recent years [1–3]. Of particular interest for the design of industrial production processes is the productivity of the process. The total productivity of the bioreactor Q_p can be expressed by the equation:

$$Q_p = q_p XV \tag{1}$$

where q_p is the specific product formation rate, X is the concentration of biomass and V is the bioreactor volume. It can be seen from equation 1 that the productivity is directly proportional to the concentration of biomass (X). The productivity can therefore be increased with the help of a high growth rate and high feed rate of the limiting substrate.

In fed-batch processes, the cell productivity and final cell concentration are limited by the rate of substrate consumption (causing engineering limitations) and the rate of maintenance q_m. Assuming that the maintenance energy reaction (q_m) [4] has priority to the available substrate, the theoretical maximum value for the total biomass $(XV)_{max}$ can be obtained by solving the mass balance equations for a fed-batch for $\mu = 0$ [5]:

$$(XV)_{\max} = \frac{F_S S_i}{q_m} \tag{2}$$

where S_i is the concentration of the feed solution and V is the volume of the bioreactor.

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The (maximum) feeding rate F_s for the energy source is limited by the oxygen and/or heat transfer capacity of the bioreactor. By increasing the oxygen transfer capacity of the bioreactor it is possible to achieve higher cell productivity and final biomass concentration.

It has been shown earlier [6] that restricted oxygen supply resulted in the excretion of several metabolites from the mixed acid metabolism, i.e. succinate, formate, acetate, lactate, ethanol and hydrogen gas. Excretion of such metabolic by-products is undesired, since the overall productivity of the bioreactor [7] and the production of a recombinant protein might be affected [8].

The oxygen transfer rate is usually controlled by the rate of agitation and the gas flow rate. It is also possible to increase the driving force of oxygen transfer by increasing the pressure in the bioreactor [9]. However, this method has the drawback that the partial pressure of CO_2 will be increased as well [10], which might have undesired effects, such as acetate production [10].

Another possibility to control the oxygen transfer rate is to increase the partial pressure of oxygen in the air supply, e.g. with oxygen enriched air. Since air separation systems based on permeable membrane gas separation technology have been developed [11], the interest in the use of oxygen enriched air has increased.

When designing a process application with oxygen enriched air, a hypothetical process may consist of three parts: First, the feed rate is increased exponentially until the oxygen concentration reaches a minimum acceptable value (measured as the dissolved oxygen tension). Second, the exponential feed continues, but the increased oxygen demand is satisfied by a gradual enrichment of the air supply with oxygen and third, the feed rate is changed to constant when the concentration of carbon dioxide reaches a maximum acceptable value. A major gain in the final biomass concentration and the productivity is so achieved, especially when the specific product formation rate is mainly growth associated.

However, when oxygen enriched air is used in order to achieve high feed rate, the impact of high oxygen concentrations needs to be investigated. Oxygen itself is potentially toxic also for aerobic microorganisms such as *E. coli* [12, 13]. The possible toxic effect of oxygen enrichment on *E. coli* was therefore investigated in this study. Furthermore, the impact of dissolved oxygen concentration on the production of recombinant proteins is ambiguous in literature. Examples for the beneficial effect of oxygen on the productivity and quality [14] can be found as well as the contrary [14,15].

Further, high feed rates of the limiting substrate result in high carbon dioxide production rates and by that high carbon dioxide concentrations in the bioreactor. The dissolved carbon dioxide concentration depends on the partial pressure of the carbon dioxide according to Henry's law. Growth inhibition and toxic effects of carbon dioxide are reported [16–18]. However, the sensitivity for different species is variable. Yeasts and lactobacilli are reported to be most resistant, while pseudomonads and moulds are most sensitive [19]. At elevated CO_2 concentrations, carboxylation alters various metabolite pools and has an impact on amino acid production. Previous efforts to use oxygen enriched air for penicillin production have failed [17,20] due to increased carbon dioxide concentrations. Compared to for example moulds, *E. coli* is less sensitive to carbon dioxide concentration [19]. Prerequisites for the use of oxygen enriched air are therefore much better in *E. coli* processes than in e.g. penicillin process. Another reason for testing oxygen enriched air in *E. coli* processes is the fact that high rates of maintenance can be observed in *E. coli* [1]. The high rate of maintenance makes the final cell concentration very much dependent on the rate of energy and oxygen supply (Eq. 2, Fig. 1).

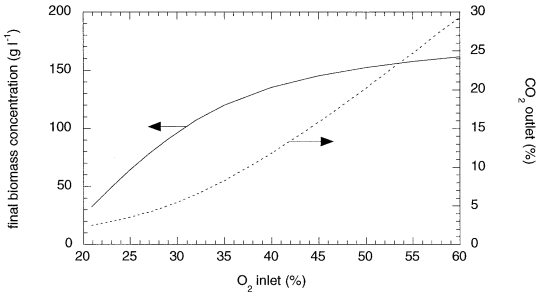

Fig. 1. Illustration of the connection between high biomass concentration and carbon dioxide concentration in cultivations with oxygen enriched air: Simulated data on the final biomass concentration (straight line) and the concentration of carbon dioxide in the out gas (dotted line). The simulations were performed according to the model equations described in [37]. The volumetric oxygen transfer coefficient was set as $k_la = 1000\ h^{-1}$, the initial volume was 3 liters. The dissolved oxygen tension was controlled to 25% by feed-back control from the dissolved oxygen concentration (DO-stat, [37,38]). The simulation was terminated when the volumetric productivity for biomass, i.e. μ*X, declined below a value lower than 4 g l^{-1} h^{-1}. The final biomass concentration and concentration of carbon dioxide at this point are shown in the figure.

A lot of basic research has been performed investigating the impact of elevated concentrations of oxygen and carbon dioxide on *E. coli* [21]. However, the application of oxygen enrichment on high cell density processes is not so well described. Since *E. coli* processes gain increasing interest for the production of high value products, such as pharmaceuticals and enzymes, the impact of elevated oxygen and carbon dioxide concentrations on the *E. coli* metabolism and on the production rate of biomass and desired product need to be investigated further.

This paper reports on the use of oxygen enrichment to achieve high productivity in *E. coli* processes with and without production of recombinant human growth hormone (rhGH).

2. Materials and methods

2.1. Organism and medium

The bacterial strain used in this study was the wild type *Escherichia coli* K-12 strain W3110. The strain was either used as a wild-type strain, containing no plasmid, or with a pBR322-derived plasmid coding for recombinant human growth hormone (rhGH). An inducible promoter was used to induce production at about 25 h. Cultivations were performed in a glucose mineral salt medium as described by [22], however minor modifications were applied for the rhGH production cultivations. Medium components together with distilled water were sterilized in the bioreactor at 121°C for 30 min. Sterilized magnesium sulfate, trace elements and the initial glucose for the biomass producing process (20 g l^{-1}) were added separately after sterilization. Breox 125 (International Speciality Chemicals, Ltd., Southampton, UK), was added as needed for foam control. The glucose feed solution for the fed-batch had a concentration of 600 g l^{-1}.

2.2. Preparation of the inoculum

The inoculum for all experiments was prepared by cultivating 200 μl from a defrosted cell bank vial in a 2 liters shake flask containing 500 ml of the above medium at 34°C for 16 h. The medium was adjusted to pH 7 before sterilization; the initial glucose concentration was 5 g l^{-1}. When the optical density reached 2 in the shake flask, 450 ml were transferred to the production bioreactors. That was a 15 liters CF 300 Chemap (Switzerland) bioreactor with a start volume of 6 liters in the rhGH process and a Belach (Sweden) 9 liters bioreactor with a start volume of 4 liters in the biomass producing process.

2.3. Cultivation procedure and bioreactors

Temperature was controlled to near the optimum growth temperature for *E. coli*, and the pH was controlled near neutrality by the addition of 25% NH_3 (aq). Dissolved oxygen tension (DOT) in the bioreactor was measured by a polarographic oxygen electrode (Ingold, Switzerland). The stirrer speed was increased to 1000 rpm during the first hours of the cultivations, maintaining a DOT above 20%. The aeration rate was 1 vvm calculated on the initial volume. No overpressure was applied. The variation in the concentration of the inlet gas was achieved by adding 100% oxygen or carbon dioxide to the airflow. Mass flow controllers were used to control the gas flow.

2.4. Analytical methods

The cell dry weight (CDW) was determined by centrifugation of 3 × 5 ml of cell suspension in preweighed tubes for 10 min at 4500 rpm, washing the pellet with 5 ml of distilled water, recentrifuging and drying overnight at 105°C before weighing. The supernatant from the first centrifugation was filtered with a 0.2 μm filter and used for DNA analysis. Samples for the analysis of acetate, formate and glucose were taken by rapid sampling into preweighed tubes containing perchloric acid, as described by [23]. Acetate, formate and glucose were analyzed using enzymatic kits (Boehringer Mannheim, Germany), kit no 148261, kit no. 979732 and kit no. 716251, respectively. Analysis of the DNA concentration in the medium was performed with the Pico-Green method [24]. The concentration of phosphate in the medium was monitored using the test kit LCK 349 from Dr Lange, Germany.

Samples for product analyses were taken from the pellet fraction of 5 ml of centrifuged cell suspension. Centrifugation was performed at 5000 rpm for 15 min at +4°C. Two extraction methods were used in order to release the periplasmic fraction of proteins:

1. Osmotic shock treatment with an extraction buffer consisting of 50% sucrose, 0.2 M Tris and 0.1 M EDTA buffer, as described by Chaib [25]. However, the method was adapted to small scale and one ml buffer was added to resuspend the pellet. The incubation conditions were 20 min at 5°C. After the incubation, the mixture was diluted with cold distilled water to a volume of 10 ml, centrifuged and the supernatant was analyzed with RP-HPLC.
2. Freezing of the pellet followed by resuspension in 5 ml extraction buffer consisting of (per liter): Tris-HCl, 0.9837 g; Tris-base, 0.4551 g; and ethylenediamine tetra-acetic acid (EDTA) × $2H_2O$, 0.372 g. The incubation condition was 30 min at 5°C. After the incubation, the mixture was centrifuged and the supernatant was analysed with RP-HPLC.

Purity and quantity of rhGH were determined by isocratic reverse-phase high-performance liquid chromatography (RP-HPLC). Three peaks (A to C) were identified and characterized with RP-HPLC [26,27]: peak A contained (Met(O)125)-rhGH and (des-Phe1, des-Pro2)-rhGH; peak B contained (Met(O)14)-rhGH, (des-Phe1)-rhGH (LMW), (clipped 142/143)-rhGH (clip-2), and deamidated rhGH; and peak C contained rhGH and (trisulfide Cys182–Cys189)-rhGH.

3. Results and discussion

3.1. Batch cultivations

The investigated concentrations for carbon dioxide were 10 and 20% and for oxygen they were 30 and 40%. The experimental domain was chosen in accordance to Fig. 1.

The effect of oxygen enrichment on the biomass concentration is highest at relatively low oxygen additions and above 40% inlet oxygen concentrations, little effect is seen.

Table 1
Maximum specific growth rate (μ_{max}), yield coefficient ($Y_{x/s}$) and acetate concentration at the end of the cultivation in *E. coli* batch cultivations with varied compositions of the inlet gas flow

Composition of the gas supply (% oxygen/% carbon dioxide)	μ_{max} (h^{-1})	$Y_{x/s}$ (g/g)	final acetate conc. (g/l)
20.95/0.033 (reference with air)	0.66	0.46	0.64
18.91/9.75	0.61	0.47	1.03
16.87/19.48	0.56	0.49	1.18
30/0.029	0.66	0.47	0.49
40 /0.025	0.66	0.46	0.76

The cell growth, glucose consumption, yield coefficient and metabolic by-product formation were analyzed. The final sample was taken exactly when the initial glucose (20 g l^{-1}) was consumed according to the signal from the dissolved oxygen electrode.

The results for this series of batch cultures with varying concentration of the gas supply are summarized in Table 1. It can be observed that the maximum specific growth rate (μ_{max}) decreased when the concentration of carbon dioxide was increased. However, increased oxygen concentrations in the gas bubbles had no inhibitory effect on the specific growth rate.

The experiments indicated clearly that the biomass yield coefficient ($Y_{x/s}$) increased when the culture was exposed to high concentrations of carbon dioxide (>20%). This increase in the biomass yield was even more surprising, since the cultivations with high carbon dioxide concentration produced significantly more acetate than the reference or the cultivations with high oxygen concentration.

The decrease of the maximum specific growth rate in the cultivations with high carbon dioxide concentrations can be seen as confirmation of negative effects of carbon dioxide on *E. coli*, as described in the literature [16,18,28]. However, the decrease in specific growth rate is not on expense of the biomass yield, which was in contrast found to increase in these experiments. That increase in the biomass yield might be due to anapleurotic reactions, i.e. carbon dioxide fixation directly into the TCA cycle [29,30]. One anapleurotic reaction is the carboxylation of phosphoenolpyruvate to form oxaloacetate. The increased excretion of acetate at high concentrations of carbon dioxide confirmed previous data [10].

3.2. Process for the production of biomass

After an initial batch phase that yielded about 10 g l^{-1} biomass, the exponential glucose feed was started and this resulted in an approximately exponential growth with a specific growth rate of about 0.25 h^{-1} (Fig. 2). When the dissolved oxygen tension reached about 20% air saturation at about 6.5 h, the feed rate was kept constant in the reference process but in the other process oxygen enrichment of the air was started. This raised the dissolved oxygen

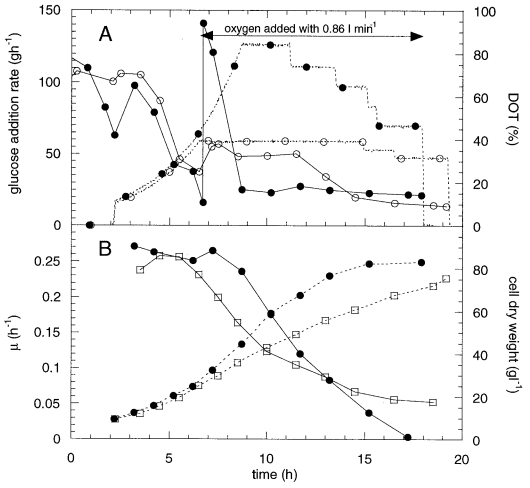

Fig. 2. Dissolved oxygen concentration, glucose feed rate (Panel A), specific growth rate and biomass concentration (Panel B) for two cultivations with *E. coli* K-12 strain W3110. The reference cultivation is represented with (□), the cultivation with oxygen enriched air with (●). In panel A, the glucose feed rate is represented with dotted lines, dissolved oxygen concentration by solid lines. In panel B, biomass concentration is represented with dotted lines, specific growth rate by solid lines.

tension to about 95% while the feed was permitted to increase exponentially until the dissolved oxygen tension reached about 20% again. Then the feed rate to the oxygen enriched process was set constant. However, the dissolved oxygen tension declined in spite of the constant feed rate. To prevent oxygen limitation, the feed rate was then gradually stepped down as indicated in Fig. 2. Also in the reference process the feed rate had to be reduced in the end, but less drastically.

The biomass concentration increased faster in the oxygen enriched process and the biomass yield (Fig. 3) dropped gradually as expected until about the 15th hour when the specific growth rate was about 0.05 h^{-1}. However, the growth rate dropped faster in the oxygen enriched process due to the reduction of the feed rate and eventually growth ceased in the oxygen enriched process (Fig. 2). Even the yield coefficient of biomass per added glucose decreased drastically after 13 h. Between 13.2 and 17.2 h, 311 g glucose were added to the culture, producing 75 g biomass, 0.6 g formate and 0.9 g acetate in the culture with oxygen enrichment, while respiration was almost constant. The yield coefficient of oxygen per glucose added ($Y_{o/s}$) increased dramatically when the growth ceased, indicating a dramatic increase in the maintenance coefficient (Fig. 4). Such an effect might be expected from e.g. phosphate limitation, but the phosphate concentration was monitored continuously and the final concentration of phosphate was 620 mg l^{-1}.

The analysis of glycolytic byproducts revealed that both higher concentrations of formate and acetate could be mea-

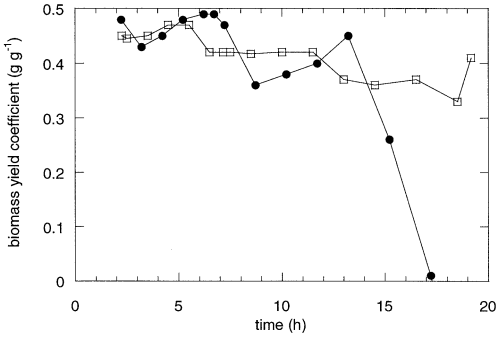

Fig. 3. Yield coefficient of biomass per added glucose for two cultivations with *E. coli* K-12 strain W3110. The reference cultivation is represented with (□), the cultivation with oxygen enriched air with (●). The oxygen enrichment was started after 6.6 h.

sured in the cultivation with oxygen enriched air (Fig. 5). In both cultures, the concentrations of acetate and formate were low until the oxygen enrichment was started. After that the excretion of both metabolites increased drastically compared to the reference. The reason for the higher excretion of products from mixed acid fermentation in the cultivation with oxygen enrichment might be found in the higher degree of cell lysis that was observed in that cultivation in the form of excessive foaming.

3.3. Process for the production of recombinant human growth hormone (rhGH)

The aim of the experiments was to double the cell mass before induction and investigate the product formation with respect to productivity and product quality. The total process time was supposed to be the same in both cultivations.

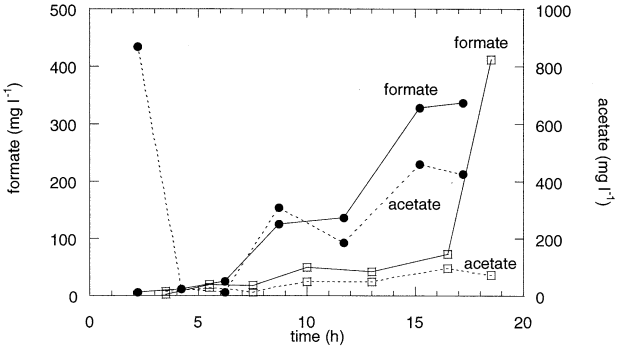

Fig. 5. Data on the concentration of acetate and formate in the cultivation medium from two cultivations with *E. coli* K-12 strain W3110. The reference cultivation is represented with (□), the cultivation with oxygen enriched air with (●). Acetate is represented by dotted lines, formate by straight lines.

Therefore, oxygen enriched air supply was used in order to be able to achieve the necessary high feed rates. The process consisted in principle of four different phases, that is one batch phase and three fed-batch phases. The glucose feed was started immediately. The excess glucose resulted in a batch phase of 2 to 4 h. After depletion of the excess glucose, the exponential growth- limited feed phase was started, resulting in the corresponding exponential growth ($\mu < \mu_{max}$). After this, the first constant feed phase started and the specific growth rate declined successively (Fig. 6), as expected, whereas the biomass increased linearly. In the cultivation with oxygen enriched air supply, the exponential feed continued until the double feed rate compared to the reference cultivation. The total biomass increased at about constant rate during the constant feed phase and reached about 77% higher value than the reference process at the time of induction (arrow in Fig. 7). Growth continued after induction, but at a low rate. The corresponding final biomass concentration in the reference cultivation was in the order of 40 g l^{-1}. Data on the cell growth and the dissolved

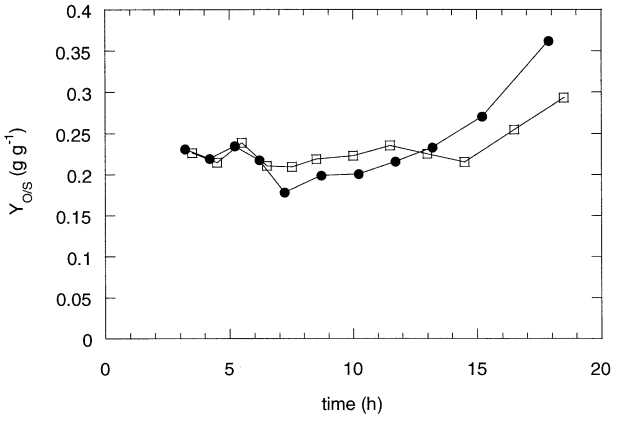

Fig. 4. Yield coefficient of oxygen per glucose added ($Y_{O2/S,}$ g g^{-1}) for two cultivations with *E. coli* K-12 strain W3110. The reference cultivation is represented with (□), the cultivation with oxygen enriched air with (■). The oxygen enrichment was started after 6.6 h.

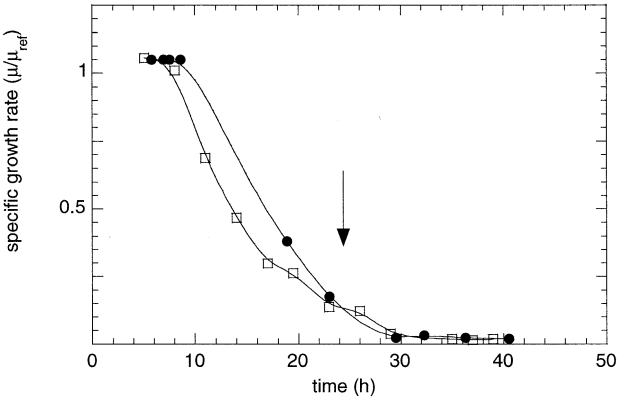

Fig. 6. Cell growth data from two cultivations with *E. coli* K-12 strain W3110 harboring a plasmid for the production of rhGH, shown as specific growth rate (μ/μ_{ref}). The reference cultivation is marked with (□), the cultivation with oxygen enriched air supply is marked with (●). The time of induction is marked with an arrow.

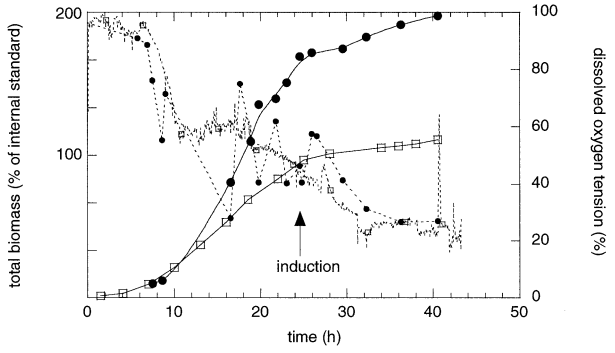

Fig. 7. Cell growth dissolved oxygen tension data from two cultivations with *E. coli* K-12 strain W3110 harboring a plasmid for the production of rhGH. The biomass is shown as percent of an internal standard (straight lines), the signal from the dissolved oxygen electrode with dotted lines. The reference cultivation is marked with (□), the cultivation with oxygen enriched air supply is marked with (●). The time of induction is marked with an arrow.

oxygen tension are shown in Fig. 7. In both cultivations, the signal for the dissolved oxygen tension was kept above 20% saturation.

The instantaneous yield coefficient of biomass per glucose added, shown in Fig. 8, was about 0.35 g g^{-1} until approximately 20 h. After this point, the yield decreased with decreasing growth rate. However, it appeared that the yield decreased somewhat faster in the cultivation with oxygen enriched air supply. This observation is supported by the fact that the final total biomass was only increased with 77%, although medium components and maximum glucose feed were doubled. The reason could be increased stress due to the high cell concentration or the high concentrations of oxygen [13].

The total yield of the product, that is the correct rhGH monomer, is shown in Fig. 9a. The product was extracted from the periplasmic space by freeze/thawing and extraction in Tris/EDTA buffer. Extraction according to the osmotic

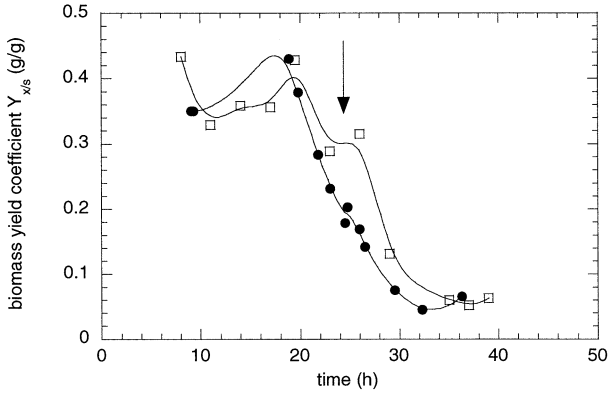

Fig. 8. Biomass yield coefficient data from two cultivations with *E. coli* K-12 strain W3110 harboring a plasmid for the production of rhGH. The reference cultivation is marked with (□), the cultivation with oxygen enriched air supply is marked with (●). The time of induction is marked with an arrow.

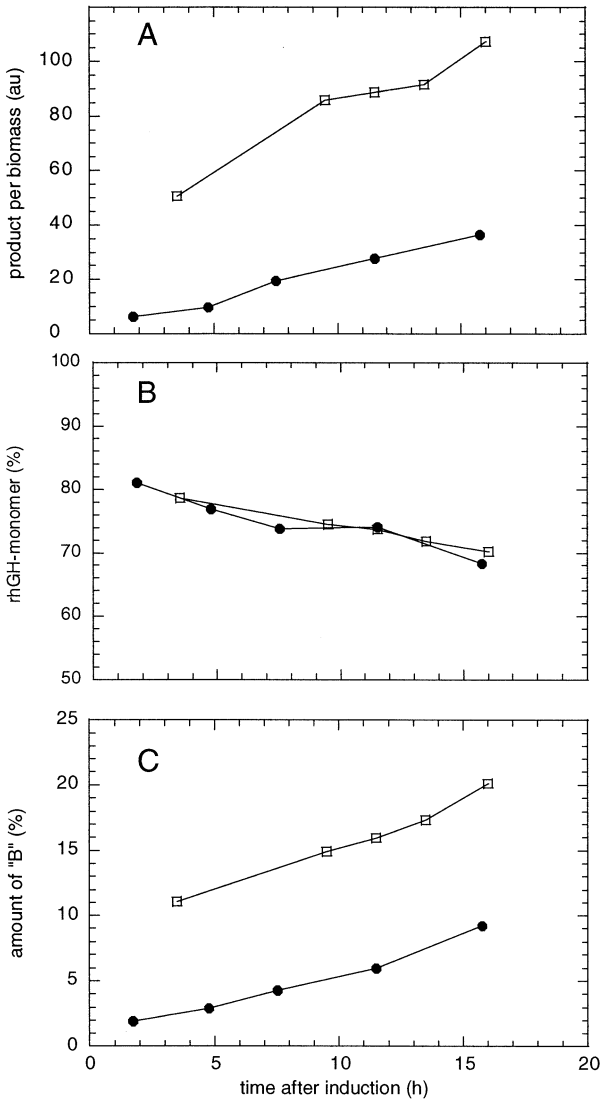

Fig. 9. Specific product data from two cultivations with *E. coli* K-12 strain W3110 harboring a plasmid for the production of rhGH. The reference cultivation is marked with (□), the cultivation with oxygen enriched air supply is marked with (●). Panel A: total amount of rhGH per biomass. Panel B: amount of correct GH monomer (% of total rhGH). Panel C: amount of "B" (% of total rhGH), which includes LMW, clip-2, (Met(O)14)-rhGH and deamidated rhGH.

shock procedure resulted in similar data to that shown in Fig. 9 (results not shown). Generally, a linear increase in the specific product concentration (g $g^{-1}_{biomass}$) is observed. However, the productivity was drastically decreased in the cultivation with oxygen enriched air supply. The final values for the specific product concentration in the two cultivations are 100 and 37 au in the reference and oxygen enriched air cultivation, respectively. The volumetric yield (g l^{-1}), however, was 58% in the oxygen-enriched cultivation, compared to the reference. When investigating the quality of the product, the amount of rhGH monomer (Fig. 9b) describes the percentage of correct GH monomer among all variants of rhGH. In Fig. 9b no significant difference in

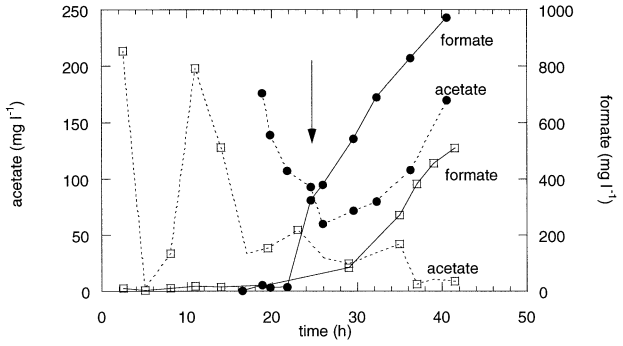

Fig. 10. Data on the concentration of acetate and formate in the cultivation medium from two cultivations with *E. coli* K-12 strain W3110 harboring a plasmid for the production of rhGH. The reference cultivation is represented with (□), the cultivation with oxygen enriched air with (●). Acetate is represented by dotted lines, formate by straight lines. The time of induction is marked with an arrow.

the amount of correct rhGH monomer between the two cultivations is shown. The amount of proteolytically degraded forms of the product was different in the two cultivations: Fig. 9c shows that the amount of peak "B" (% of total rhGH), which includes LMW, clip-2, (Met(O)14)-rhGH and deamidated rhGH, was significantly lower in the cultivation with oxygen enriched air. The final values were 20 and 9% in the reference and the cultivation with oxygen enriched air, respectively. It appeared that a cultivation technique with oxygen enriched air resulted in less proteolytical variants of the product. This can be explained by previous observations, that low product formation rates resulted in less proteolytic degradation of the rhGH (results not shown). The amount of peak "A" was only some percent in both cultivations (results not shown). However, other, not characterized variants of the product are produced in the cultivation with oxygen enriched air, since the total sum of all variants (peak A to C) should be near 100%. It can therefore be said, that the product yield declined but the quality of the product increased with respect to the content of proteolytically degraded variants (peak B) when oxygen enriched air is used.

When *E. coli* cultures are exposed to gradually increasing degree of oxygen limitation, mixed acid fermentation products (acetate, ethanol, formate, hydrogen gas, lactate, succinate) appear [6,31]. Of these products only acetate is also produced when *E. coli* is grown aerobically in the presence of excess glucose (overflow metabolism) [32]. Formate is not consumed as fast as acetate and other anaerobic products when restoring high oxygen level [33]. Thus formate can be used as an indicator for the existence of zones in the bioreactor where oxygen limitation occurs. Acetate and formate were produced in both cultivations (see Fig. 10) in spite of a value of the dissolved oxygen tension above 20% air saturation. Acetate was present during the batch phase and during the exponential phase in both cultures, but consumed during the constant feed. After induction, however, the concentration of acetate increased again in the oxygen enriched cultivation in contrast to the reference. The reason for acetate production in the first phases of the cultivation is overflow metabolism. The production of acetate after induction, however, was due to mixed acid fermentation since also formate was produced. Formate was produced also in the reference process, though at a later stage. The final formate concentrations were 500 and 1000 mg l^{-1} in the reference and oxygen enriched air cultivation, respectively. There are two possible mechanisms by which oxygen limitation is created in spite of a high DO signal: Oxygen limitation zones in the bulk liquid due to insufficient mixing and oxygen diffusion hindrance at the cell surface.

Although sufficient oxygen supply was measured throughout the cultivation with oxygen enriched air (see Fig. 7), accumulation of mixed acid fermentation products appeared. Formate concentrations of 1000 mg l^{-1} have previously been measured in similar cultivations, but this was either in large scale bioreactors or in a scale down reactor with a controlled zone of oxygen limitation [34]. An alternative mechanism of formate production in lab scale reactors has been suggested [35,36]: DNA released by cell lysis could disturb the mass transfer of oxygen to the cell, explaining formate production under aerobic conditions. This might be the reason for the production of mixed acid fermentation products, since increased cell lysis was observed in the cultivation with oxygen enriched air (results not shown). It can be speculated, that one effect of the potential oxygen toxicity might be increased cell lysis and thereby formate excretion.

4. Conclusions

Relatively low oxygen enrichment (40%) could be used to increase the biomass concentration considerably in *E. coli* fed-batch cultures. This oxygen enrichment caused several physiological effects: The biomass yield on glucose dropped faster than in the control and the oxygen consumption per glucose increased more, indicating an increasing maintenance. Furthermore, formic acid accumulated to higher concentrations. The chance for improving the performance of a process by enrichment of the air supply with oxygen is better when the product formation is growth associated. This indicates that recombinant processes need to be investigated from case to case. The multiple physiological effects by increasing the oxygen concentration to 40% makes it interesting to investigate even processes with lower oxygen enrichment.

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