On-line immunoanalysis of monoclonal antibodies during a continuous culture of hybridoma cells

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Received 28 August 1995; accepted 5 March 1996

Key words: fluidized-bed reactor, monoclonal antibody, on-line monitoring, sample system, temperature

Abstract

The monoclonal-antibody production of an immobilized hybridoma cell line cultivated in a fluidized-bed reactor was monitored on-line for nearly 900 h. The monoclonal antibody concentration was determined by an immuno affinity-chromatography method (ABICAP). Antibodies directed against the product, e.g. IgG, were immobilized on a micro-porous gel and packed in small columns. After all IgG present in the sample was bound to the immobilized antibodies, unbound proteins were removed by rinsing the column. Elution of the bound antibodies followed and the antibodies were determined by fluorescence. The analytical procedure was automated with a robotic device to enable on-line measurements. The correlation between the on-line determined data and antibody concentrations measured by HPLC was linear.

A sampling system was constructed, which was based on a pneumatically actuated in-line membrane valve integrated into the circulation loop of the reactor. Separation of the cells from the sample stream was achieved by a depth filter made of glass-fibre, situated outside the reactor. Rapid obstruction of the filter by cells or cell debris and contamination of the sample system was avoided by intermittent rinsing of the sample system with a chemical solution. The intermittent rinsing of the filter, which had a surface of 4.8 cm², resulted in an operational capacity of up to 40 samples (1.0 l total sample volume). Both the sampling system and the analytical device functioned without failure during this long-term culture.

The culture temperature was varied between 34 and 40 °C. Raising the temperature from 34 up to 37 °C resulted in a simultaneous increase of growth and specific antibody production rate. Specific metabolic rates of glucose, lactate, glutamine and ammonium stayed constant in this temperature range. A further enhancement of temperature up to 40 °C had a negative effect on the growth rate, whereas the specific monoclonal antibody production rate showed a small increase. The other specific metabolic rates also increased in the temperature range between 38 to 40 °C.

Introduction

Glycoproteins and antibodies are often produced using short-term batch cultures of animal cells. Batch pro-

duction processes have the disadvantage that the period of high cell concentrations coupled to a high productivity is used inefficiently. A prolonged use of high cell density for production, e.g. by fed-batch or continuous cultures, could increase the efficiency. During long-term production processes however, genetic instability

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of the cell line can have a negative effect on the product quantity (Jo *et al.*, 1993) and/or quality, e.g. glycosylation patterns (Robinson *et al.*, 1994), when no selection pressure exists (Werner *et al.*, 1992). On-line monitoring of the product using a highly selective assay will create the possibility of avoiding inefficient production periods by detecting alterations in product quality and quantity. Moreover, on-line monitoring will be useful for validation purposes.

To measure high-molecular weight products of animal-cell cultures, antibodies are normally used in standard methods as the Enzyme-Linked Immunosorbent Assay (ELISA). ELISA can be adapted for on-line monitoring by combining immobilized antibodies and Flow Injection Analysis (FIA). With this combination, it is not necessary to reach a binding equilibrium between antibody and antigen, because of the highly reproducible reaction times of FIA (Mattiasson *et al.*, 1989). This feature considerably shortens the assay time. A disadvantage of the automated method is the inactivation of the immobilized antibodies during dissociation of the bound antigen from the immobilized antibodies (Nilsson *et al.*, 1993). Gebbert *et al.* (1994) compensated for the effect of inactivation by regular calibration during the on-line determination of IgG with a combination of a sandwich-ELISA method and FIA.

Another on-line assay applied, is the competitive method, which is based on competition for binding places of the immobilized antibodies between previously mixed labelled antigen and native antigen of the sample. Inactivation of the bound antibodies will influence the amplitude of the response of this method but not the ratio between labelled antigen and native antigen (Mattiasson and Hakanson, 1992). The inactivation can be corrected by intermittent calibration with only labelled antigen. The competitive method was used to measure products such as proinsulin (Birnbaum *et al.*, 1986), pullulanase (Degelau *et al.*, 1992), IgG (Stöcklein and Schmid, 1990) and α -amylase (Nilsson *et al.*, 1993). A disadvantage of competitive assays is that labelled antigen must be available and that the labelled antigen can be instable (Gebbert *et al.*, 1994).

The changing capacitance of a surface with immobilized antibodies was used to determine on-line the IgG concentration (Gebbert *et al.*, 1994). This direct measurement has the advantage that no additional reactions are necessary. However, regular calibration is still required to compensate for the inactivation of immobilized antibodies.

A different approach is the measurement of IgG through the formation of immuno precipitates between antibody and antigen (Worsfold *et al.*, 1985). Freitag *et al.* (1991) adapted this method for monitoring on-line the concentration of pullulanase and anti-thrombin III. The sensitivity of this method is not as high as the automated ELISA, but is sufficient to measure product concentrations during the culture period.

A commercially available method, ABICAP (Antibody Immuno Column for Analytical Processes), was used to determine on-line the Monoclonal Antibody (MAB) concentration of a hybridoma culture. The hybridoma cells were immobilized on microporous carriers and cultured in a fluidized-bed reactor. On-line measurements were achieved by automating the analytical method with a robotic device. A new sample system was developed to enable the on-line measurements. This sample system consisted of a pneumatically actuated in-line valve in combination with a depth filter situated outside the reactor.

During a culture period of approximately 900 h, steady-state values were determined in the temperature range of 34.5 to 40 °C. The steady-state values of the specific MAB production, growth rate and metabolic rates of the immobilized cells were compared with data from batch cultures with suspension cells (Barnabé and Butler, 1994, Bloemkolk *et al.*, 1992, Sureshkumar and Mutharasan, 1990).

Material and methods

Chemicals

All chemicals were of analytical grade. All solutions were made with distilled/de-ionized water.

The culture system

The cells were cultivated in a fluidized-bed reactor with a total volume of 350 ml (Fig. 1). The dissolved-oxygen concentration and pH were computer controlled at 80% of air saturation and 7.2 respectively, by adjusting the gas flow of air, oxygen and carbon dioxide with mass-flow valves (Brooks, Veenendaal, NL). The control program (MEAS) was developed in Forschungszentrum Jülich GmbH. Gas exchange took place by diffusion across a silicon tube integrated into the circulation loop. The temperature of the reactor was measured with a Pt-100 probe (Lauda, Lauda-Köningshöfen, FRG), placed just above the microcarrier bed. The Pt-100

Figure 1. Flow diagram of fluidized-bed reactor. Pt 100 = temperature probe, p = peristaltic pump.

probe was connected with the controller of the water-bath (Lauda) which was set to the selected temperature.

The cell strain was a gift from Merck (Darmstadt, FRG). The mouse/mouse hybridoma cell line produced an IgG_{2a} antibody (MAB 425, Murthy *et al.*, 1987) directed against the receptor for the human epidermal growth factor. The cells were negative in periodic mycoplasma tests (Boehringer, Mannheim, FRG). The cells were immobilized on porous glass carriers (Siran^R, Schott, Mainz, FRG) made of borosilicate glass. The surface of the microcarriers was modified with gelatine by an adsorptive process (Lüllau *et al.*, 1992). The total carrier volume was 85 ml. The medium as described by Van der Pol *et al.* (1994) was supplemented with 1% (v/v) heat inactivated Fetal Calf Serum (Gibco, Eggenstein, FRG).

Sample system

The sample system (Fig. 2) consisted of a stainless-steel pneumatic valve (Schico, München, FRG) inte-

grated into the circulation loop of the fluidized-bed reactor, and a cell-separation unit placed outside the reactor. Cells were separated from the sample in a filtration unit made of Teflon (TPB 025, Amafilter, Langenhagen, FRG) which contained a depth filter made of borosilicate glass (SM 13430, Sartorius, Göttingen, FRG). The depth filter had no defined pore size and is normally used as prefilter. The filter surface was 4.8 cm². The filtration unit and connecting tubes were rinsed with a sterilisation and cleaning solution before and after each sampling. The solution consisted of 0.5 M NaOH, pH 13.5, 5 mM EDTA and 0.2 M K₂S₂O₈. An electrically-driven tube-squeeze valve (Schramme, Markdorf, FRG) blocked the tube of the solution during the sampling. The sample was withdrawn by a peristaltic pump (U101, Watson Marlow, Falmouth, GB). The sample hole of the pneumatic, in-line valve was sealed by a membrane made of Ethylene-Propylene-Diene-Monomere (EPDM). All connecting tubes were made of Teflon with an inner diameter of 0.8 mm. The volume of the tubes and filtration unit was

Figure 2. The sample system. The in-line valve was integrated into the circulation loop of the fluidized-bed reactor (Fig. 1). The sampling procedure started by rinsing the sample system with the wash solution. After closing the tube-squeeze valve V2, the electrically driven valve V1 was activated so as to open the sample hole. Then peristaltic pump P withdrew medium out of the reactor. After the sample system was rinsed with medium and a sample was taken for analysis, the sample hole was closed by inactivating of valve V1. The sample procedure was ended by rinsing of the sample system with the wash solution.

approximately 4 ml. The hardware components were controlled by the process computer.

On-line determination of the monoclonal antibody concentration

The on-line measurements were made with the ABICAP system (Abion, Jülich, FRG). Antibodies directed against the produced monoclonal antibodies were immobilized on a microporous gel (not specified by the producer). The gel matrix was packed into small polystyrol columns, which contain a porous filter at the bottom. A second filter was positioned at the top. Each column was used only once to avoid any influence of inactivated immobilized antibodies on the reproducibility of the assay. The small diffusional pathways in the gel, in combination with the excess of binding sites for the analyte, resulted in an endpoint assay (Hartmann *et al.*, 1993). The detection range was 5–500 mg l⁻¹.

The measurements were automated with an ASPEC (Automatic Sample Preparation with Extraction Columns) automate (Gilson, Langefeld, FRG) (Fig. 3). The sample was pumped through a small box, which was closed on the uppermost side by a septum. After the complete sample system was rinsed

with 25 ml of sample solution, a needle on a robotic arm passed through the septum and 500 µl of sample was withdrawn using a syringe (401, Gilson). After the sample was introduced on and passed over an ABICAP-column by gravitation, unbound proteins were removed from the column by rinsing three times with a wash solution. The wash solution consisted of 50 mM tris(hydroxymethyl)-aminomethane, pH 8.0 and 150 mM NaCl. The wash cycles were followed by elution of the bound monoclonal antibodies off the column with a buffer, which consisted of 100 mM glycine, pH 2.7 and 0.15 M NaCl. The eluent was collected in a test tube, drawn up through the needle on the robotic arm and transported to an injection valve (Rheodyne, Cotrati, USA). After the injection coil was filled, the eluent was injected into a carrier flow and transported to a fluorescence detector (RF-551, Shimadzu, Duisburg, FRG). Elution buffer was used as carrier solution. The carrier flow (0.4 ml min⁻¹) was accomplished by a low-pulse piston pump (Dosimat E665, Methrom, Filderstadt, FRG). The excitation and emission wavelength of the fluorescence detector were set to 280 and 340 nm, respectively.

One complete measurement consisted of measuring a sample, original medium (blank) and 0.33 µg ml⁻¹ BSA dissolved in the wash solution.

Figure 3. The automation of the ABICAP method with an ASPEC automate. The injection needle can be moved vertically and is placed on a robotic arm, which moves freely in a horizontal plain. The measurement starts with the withdrawal of 500 μ l of sample by the injection needle. The injection needle is then placed above on an ABICAP-column by the robotic arm, and the sample is brought on the column. After the injection needle and connective tubes are rinsed with wash buffer by the dilutor, wash buffer is given on the column through the injection needle to remove unbound proteins. This cycle is repeated with elution buffer, whereby the eluted antibodies are captured in test tubes. The eluent is withdrawn out of the test tubes through the injection needle and transported to the injection valve (IV), followed by injection into a carrier flow of elution buffer (0.4 ml min^{-1}). Peak detection is carried out with a fluorescence detector. The procedure was computer controlled. FV = 4-way valve, TV = 3-way valve, P = peristaltic pump.

The BSA measurement was used to correct for small changes of the analytical device, for example drifting of the fluorescence detector, during the long-term culture. The maximum peakheight of the sample measurement was normalized with the following equation:

$$NF = \frac{P_{\max_{product}} - P_{\max_{blank}}}{P_{\max_{BSA}}} \quad (1)$$

NF is normalized fluorescence, $P_{\max_{product}}$ is the maximum peak height of the sample, $P_{\max_{blank}}$ is the maximum peak height of the blank and $P_{\max_{BSA}}$ is the maximum peakheight of the BSA solution.

The MAB concentration was calculated with the normalized fluorescence according to a calibration curve. Only one calibration was necessary as a con-

sequence of the endpoint assay and the normalization of the fluorescence signal.

The analysis was started by a signal from the process computer. The analysis cycle, inclusive processing of the peaks, were computer controlled using Datensystem DS450-MT 2 (Kontron, Stuttgart, FRG) as software program. One complete measurement took 6.5 min. The sample frequency was, directly after a change of the temperature, once per hour, and decreased after 8 h gradually to once per day when steady state was reached.

Off-line determinations

Monoclonal antibodies

Off-line measurements of the MAB concentration were carried out with HPLC (2156, Pharmacia LKB, Freiburg, FRG), using a ProAnaMab column (Biolytica, Lund, Sweden). 0.5 ml of sample solution was injected in a 0.11 M sodium acetate binding buffer, pH 5.0. The bound MAB's were eluted with a 0.155 M glycine, pH 1.6 buffer. Peaks were detected at 280 nm using an UV detector. The determined concentrations were corrected for the antibody concentration in the culture medium by measuring the original medium.

Cell counts

The concentration of cells in suspensions were determined using the erythrosine B exclusion method (Philips, 1973).

The concentration of immobilized cells was determined at the end of each steady state. A sample volume of approximately 0.5 ml micro-carrier was taken out of the reactor. The medium was quickly withdrawn and 2 ml 0.1 M citrate buffer in Phosphate-Buffer-Saline (PBS), pH 7.2 was added. PBS consisted of 137 mM NaCl, 27 mM KCl, 8.1 mM Na₂HPO₄ and 1.5 mM KH₂PO₄. The microcarriers were shaken vigorously and incubated at 37 °C. After 1 h of incubation, the cells in suspension were counted with erythrosine B. The microcarriers were washed with water and dried. The volume of the carrier was calculated using a calibration curve (data not shown).

The number of lysed cells was determined indirectly (Wagner *et al.*, 1992) by measuring the activity of lactate dehydrogenase in the medium according to Vassault (1974).

Glutamine

The glutamine concentration was determined by HPLC (Hewlett Packard, Waldbronn, FRG) using derivatisation with o-phthalaldehyde (Hewlett Packard). Derivatised glutamine and other amino acids were separated on a reverse-phase column (RP-18 Hypersil ODS, Machery and Nagel, Düren, FRG). The amino acids were eluted with a 20 to 80% (v/v) gradient of methanol in 0.1 M sodium acetate. Peaks were detected with a fluorescence detector (excitation 230 nm, emission 455 nm).

Precipitation of proteins before analysis was carried out by adding 0.1 ml of trichloroacetic acid (36% (w/v)) to 0.5 ml of sample. After centrifugation, 15 µl of 4.5 M NaOH was added to 300 µl of supernatant. The sample was further diluted with PBS buffer to the selected concentration.

Ammonium

The reaction of glutamate dehydrogenase as described by Bergmeyer *et al.* (1985) was used to measure the ammonium concentration. Glutamate dehydrogenase (EC 1.4.1.3) type III from bovine liver was purchased from Sigma (Deisenhofen, FRG).

Glucose and lactate

Both the glucose and lactate concentrations were determined with an YSI 2000 automate (Yellow Springs, USA).

Results and discussion

On-line analysis and sample system

To monitor on-line high-molecular proteins, the interface between the culture and the on-line analysis, the sample system, must have no influence on the concentration of the analyte nor endanger the sterility of the culture. In addition, separation of cells and the analyte must be carried out to prevent pollution of the analytical device and/or to prevent interference by intracellular product. For low-molecular compounds, microfiltration membranes are normally applied. The suitability of such membranes for sampling of high-molecular compounds, e.g. monoclonal antibodies, was tested in a preliminary investigation. Several microfiltration membranes were applied within a cross-flow sample module, whereby the monoclonal antibody concentrations before and after filtration were measured. All the tested membranes showed a declining permeability for the antibodies with increasing filtrated volume (data not shown). This change of the filtration characteristics is due to membrane fouling (Schügerl, 1993), which causes a build-up of a protein and/or cell layer on the membrane surface. This layer reduces the effective pore size and consequently lowers the permeability for high-molecular compounds (Scheper, 1991). Yet, membrane fouling can be avoided by applying high shear forces over the membrane surface. In this way,

Figure 4. Comparison of on-line measured MAB concentration (ABICAP) with off-line HPLC measurements. The regression line has a slope of 0.944 with an intersection of 2.73 mg l^{-1} . The correlation is 0.900.

Table 1. Testing of the filtration characteristics of the depth filter with filtrated sample volume. The MAB concentrations were determined in samples taken directly out of the reactor (before filtration) and on-line (after filtration)

Sample number	Filtrated sample volume l	MAB concentration before filtration mg l^{-1}	MAB concentration after filtration mg l^{-1}
6	0.150	21.5	22.2
14	0.350	20.9	20.0
18	0.450	23.8	23.0
19	0.475	21.7	22.2
30	0.750	20.0	23.9
36	0.900	22.5	20.4
38	0.950	21.2	22.4
40	1.000	22.8	19.9

microfiltration membranes could be used for on-line monitoring of proteins (Stamm *et al.*, 1989, Freitag *et al.*, 1991). This is, however, less desirable for animal-cell cultures as a result of the damaging effect of shear forces on animal cells.

An alternative way to reduce membrane fouling is regular cleaning and/or exchange of the membrane.

This is, however, not possible as long as the membrane is in direct contact with the culture. Therefore, an in-line valve was placed between the culture and the membrane (Fig. 2). Contamination of the reactor was prevented by this in-line valve in combination with a chemical barrier. The placement of the filter behind the in-line valve enabled the intermittent cleaning of the

filter with the chemical barrier in order to remove protein and/or cell layer without endangering the sterility of the culture. Since the membrane was only used for cell filtration and not as a sterile barrier, a filter with a larger pore size than microfiltration membranes could be applied to diminish size-exclusion effects. Therefore, a depth filter designed to adsorb high quantities of particles was tested. This filter separates the cells and cell debris from the sample across the membrane instead of at the surface as is the case with microfiltration membranes. Regular microscopic control of the filtrate during the culture showed no leakage of cells. A maximum of 75 ml of sample volume (3 samples) could be filtered before the depth filter became blocked. To increase the operational capacity of the filter, several sterilization solutions and additives were tested for their effectiveness in cleaning the filter between sampling. A 0.5 M sulphuric acid solution caused rapid sticking of the filter. A 0.5 M NaOH solution with additional EDTA, to prevent precipitation of carbonate salts, extended the operational capacity. A further increase was achieved by adding an oxidizing agent, potassium peroxodisulphate, to the sterilization solution. This resulted in an operational capacity of up to 40 sampling cycles (1.0 l sample volume) per filter.

To detect possible retention of the MAB's by the filter, on-line measured data were compared with antibody concentrations measured in off-line samples taken directly out of the reactor. As can be seen in Table 1, the filter showed an unbiased permeability for antibodies after 40 samples, which were taken on-line over a period of 10 d.

The ABICAP method combines the high-binding capacity of immobilized antibodies with the very short diffusion pathways in a gel matrix. The short diffusion times result in a fast adjustment of the binding equilibrium between antibody and antigen. All assay designs known for ELISA can be adapted to this method (Hartmann *et al.*, 1993). The ABICAP method was evaluated by comparison of the on-line data with off-line measured MAB concentrations, which were determined by HPLC (Fig. 4). Linear regression between off-line and on-line data resulted in a slope of 0.944 with an intersection at 2.73 mg l^{-1} . The correlation was 0.900.

Using a new column of immobilized antibodies for every measurement eliminated any influence that inactivation of the immobilized antibodies may have had on the assay. It also simplified the analysis method considerably, as no labelled antigen or frequent recalibration was necessary. The analytical device functioned without any failures during the culture of 900 h. The fluo-

rescence detector showed a small drift in time, which was corrected by the BSA measurement.

Influence of temperature on the cell metabolism

The relatively high analysis frequency (once per hour) after a temperature change revealed that the MAB concentration can change quickly (Fig. 5). The MAB concentration can also be a sensitive parameter to indicate an alternation of the cell metabolism. Yet, the MAB concentration results from the influence of the environment on the cells and does not elucidate the cause of the change. A good example of this can be seen in Fig. 5, after the temperature was enhanced from 39 to 40 °C. The MAB concentration increased simultaneously with the LDH concentration after this change of temperature. The fast increasing LDH concentration indicates the lysis of many cells (Goergen *et al.*, 1993), which may result in the release of intracellular antibodies and the peak of MAB concentration.

The growth rate was calculated using the mass balance over the reactor for suspension cells released from the microcarriers and lysed cells, and the mass balance of cells over the microcarriers. The calculated growth rates were, however, too high (data not shown). It appeared that with only one incubation with citrate buffer, as used in this study, not all cells were released from the microcarriers. However, the determined immobilized-cell concentration were constant for the temperature range from 34.5 to 39 °C. This corresponds with results of Thömmes *et al.* (1993), who also found a constant degree of colonization under different culture conditions. Only at 40 °C, the immobilized-cell concentration decreased significantly by 50%. Therefore, the trend of the immobilized-cell concentration are considered the same and the growth and other specific metabolic rates are presented as a percentage of the specific rates at 37 °C (Fig. 6).

The specific MAB production and the growth rate simultaneously increased from 34 up to 37 °C. A further increase in temperature had the opposite effect, as the growth rate decreased and the specific MAB production further increased to a maximum at 40 °C. Different optimal temperatures for maximum specific production rate and growth rate are also found for suspension cells (Table 2). Barnabé and Butler (1994) suggested that changes in specific nucleotide concentrations and ratios may have an influence on the specific production rate. The maximum MAB concentration of 25 mg l^{-1} was however reached at 38 °C (Fig. 5),

Figure 5. Course of the concentration of MAB (a) and LDH (b) during culture.

because the immobilized cell concentration at this temperature was twice as high as at 40 °C (data not shown).

The specific consumption rate of glucose (q_{glc}) and glutamine (q_{gln}) and the specific production rate of lactate (q_{lac}) and ammonium (q_{amm}) were not influenced by raising the temperature from 34 to 38 °C. At higher temperatures up to 40 °C, all four rates increased

simultaneously, which correspond with data from suspension cultures (Table 2). The yield of moles lactate produced per mole glucose consumed ($Y_{\text{lac}/\text{glc}}$) stayed constant at 1.31 ± 0.07 in the investigated temperature range, which was also found by Barnabé and Butler (1994), but this contradicts data from Sureshkumar and Mutharasan (1990). The yield of moles ammo-

Figure 6. Steady-state metabolic rates at different temperatures. The metabolic rates are in percentages of metabolic rate at 37 °C (100%) (a) Growth rate (○) and specific MAB production rate (□). (b) Specific glutamine consumption rate (▲) and specific ammonium production rate (◇). (c) Specific glucose consumption rate (●) and specific lactate production rate (▽).

nium produced per mole glutamine ($Y_{amm/gln}$) consumed showed some, but not consistent variation, and is therefore regarded as constant at 0.35 ± 0.06 for the investigated temperature range. The constant yield of ammonium from glutamine partly agreed with data from Barnabé and Butler (1994), who found a constant yield for the temperature range between 37–39 °C

Conclusions

A sample system with a combination of a chemical barrier and cell filtration outside the reactor was successfully used to monitor on-line the antibody production of a hybridoma culture for almost 900 h. The chemical barrier enabled intermittent rinsing of the filter to eliminate the influence of membrane fouling on the permeability for antibodies. Moreover, it enabled a further reduction of size-exclusion effects by the application of a depth filter with larger pore size than a microfiltra-

Table 2. The temperature (range) at which maximum specific metabolic rates were reached for suspension cultures and immobilized culture

cell line	temperature range	q_{mab}	μ	q_{glc}	q_{lac}	$Y_{\text{lac}/\text{glc}}$
<i>Suspension cultures</i>						
murine ¹	33–39 °C	39 °C	33–37 °C	39 °C	39 °C	constant
rat-mouse-mouse ²	34–38 °C	constant	34–37 °C	n.d.	n.d.	n.d.
mouse-mouse ³	33–39 °C	39 °C	35–39 °C	39 °C	39 °C	39 °C
<i>Immobilized culture</i>						
mouse-mouse	34–40 °C	40 °C	37 °C	40 °C	40 °C	constant

n.d. = not determined, q_{mab} = specific MAB production rate; μ = growth rate; q_{glc} = specific glucose consumption rate; q_{lac} = specific lactate production rate; $Y_{\text{lac}/\text{glc}}$ = ratio of mol produced lactate to mol consumed glucose. ¹ Barnabé and Butler (1994), ² agitated cultures, Bloemkolk *et al.* (1992), ³ Sureshkumar and Mutharasan (1990).

tion membrane. This resulted in a filtration capacity of 1.0 l sample volume (40 samples) or more per membrane. The automated ABICAP method proved to be very reliable and accurate in comparison with HPLC. With a combination of the ABICAP method and the sample system, it will be possible to detect deterioration of the production caused by genetic alteration of the cells. This opens a possibility to intervene in the production process at an early stage to avoid inefficient production.

The maximum specific MAB production rate and the maximum growth rate of the immobilized hybridoma cell line were reached at 40 and 37 °C respectively. The maximum specific metabolic rates for glucose, glutamine, lactate and ammonium were reached at 40 °C, whereas $Y_{\text{lac}/\text{glu}}$ and $Y_{\text{amm}/\text{glu}}$ stayed constant for the investigated temperature range. The influence of temperature on the specific metabolic rates of immobilized cells and suspension cells was similar.

Acknowledgements

The authors want to thank Ms. Cornelia Herford of the Forschungszentrum Jülich for the glutamine analysis and Dr. Allison Reid of Shell International, The Hague for correcting the spelling.

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