Cytotechnology 7: 63–74, 1991.

The medium cycle bioreactor (MCB): Monoclonal antibody production in a new economic production system

R. Kempken, H. Büntemeyer and J. Lehmann
Institute for Cell Culture Technology, University of Bielefeld, P.O. Box 8640, 4800 Bielefeld 1, Germany

Received 25 March 1991; accepted in revised form 29 August 1991

Key words: economy, medium, recycling, re-use, supplementation, hybridoma, antibody production, productivity, protein retention, substrate utilization, amino acids

Abstract

The perfusion mode of a continuous cell culture bioreactor was modified to establish a closed loop system. Eighty percent of the spent medium was re-used twice. The medium cycle bioreactor unit was operated sterile and uncomplicated without a technical retention system for the high molecular weight substances. Therefore, only 20% of the actual medium was necessary to run the recycling process. During seven days culture time in a two liter scale 5 grams of IgG_1 type monoclonal antibody was produced. During that period the cell specific productivity was constant. Renewal of proteins was omitted because the protein content in the system persisted at a high level. Therefore, self-conditioning substances of the cells were retained in the system as well as the expensive medium components (proteins with catalytic or stimulating function). Seventy to 80% of medium costs and medium quantity were saved for each medium recycling step. Only cheap metabolites that are consumed by the cells had to be supplemented. Uptake rates of glucose and amino acids were calculated to establish a suitable supplementation mixture for the recirculated medium.

Introduction

Production processes with cell cultures rank with the most expensive procedures in biotechnology. Now as before the upstream processing cause the main part of the process costs. Mammalian cell culture is rather uneconomical, because the major part of the medium is not exploited, but wasted. In respect to a more economic use of culture process components and a reduction of environmental burden it is advantageous to re-use the spent medium. Re-use of a serum-containing medium was described by Mizrahi and Avihoo (1977). This paper presents a new procedure with a serum-free medium, the combination of cell retention and continuous medium recirculation (Kempken et al., 1991a).

Development of the medium cycle bioreactor

Cell retention in the bioreactor

Cell retention became rationalised for the slow growth rate of mammalian cells. Synthetic membranes are used in numerous separation problems

in biotechnology (Chmiel et al., 1985) and enable a reliable cell retention by microfiltration of the bioreactor fluid. Concerning cell cultures, systems were developed dealing with medium filtration inside the bioreactor. Double membrane stirrers are very proper for this (Büntemeyer et al., 1987). A hydrophilized polypropylene membrane is used for long-term filtration (Lehmann et al., 1988). This kind of cell retention permits an effective perfusion mode in homogeneous continuous systems (Büntemeyer et al., 1990) resulting in reproducible steady state cultivation (Büntemeyer et al., 1991).

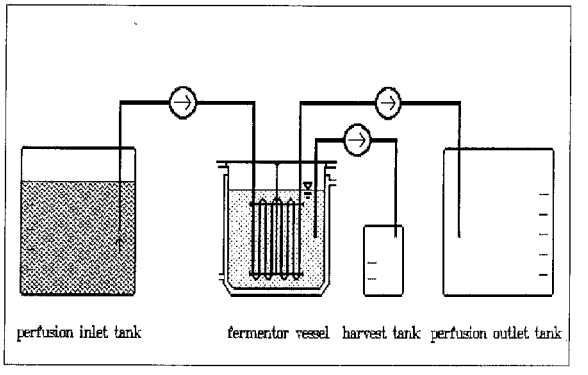

Fig. 1. MCB status at the start of the perfusion period.

Recycling of the cell culture medium

For the preceding arguments medium recycling should be an advantageous completion of the perfusion mode. We recommend a loop or a bypass system which passes the major part of the spent medium back into the bioreactor. A small part should be wasted as usual to avoid an increase of toxic metabolites that are formed by the cells. Hence, a dilution step and a supplementation of consumed nutrients are necessary to run the recycling procedure (Lehmann et al., 1989). A continuous perfusion mode with subsequent ultrafiltration module for example allows an online recirculation of the high molecular weight medium components (Büntemeyer, 1988). The partition of the bioreactor outlet fluid in high and low molecular weight branches can be extended to an overall conception of product harvest and inhibitor removal in the protein branch, and detoxification and nutrient supplementation in the protein-free branch (Lehmann et al., 1989). For the feasibility of such recirculation processes no loss of productivity must occur plus quality, stability and purity of the product must be maintained. Hence, we have to investigate whether an accumulation of substances with inhibiting or toxic effects is found in respect to the re-use of spent media. A detoxification of such substances on the low molecular side was performed for example by Rønning and Schartum (1990) with gel filtration, and by our group with membrane extraction (Lütkemeyer et al., 1991) and electrodialysis (Kempken et al., 1990a). We have no reliable findings about structure and effect of high molecular weight inhibitors yet. For these considerations the medium cycle bioreactor was developed essentially as a system for the accumulation and discovery of possible inhibitors.

Principle of the medium cycle bioreactor

Using the medium cycle bioreactor, the perfusion mode is extended to form a closed loop system. Batch and perfusion start in the same way as in classic perfusion mode (Fig. 1). Eighty percent of the perfused medium is collected cell-free as perfusion outlet fluid and 20% as cell-containing bleed. When the medium inlet tank is empty (Fig. 2), the usual procedure is to take the product out of bleed tank and perfusion outlet tank and to continue the culture with new medium. On the

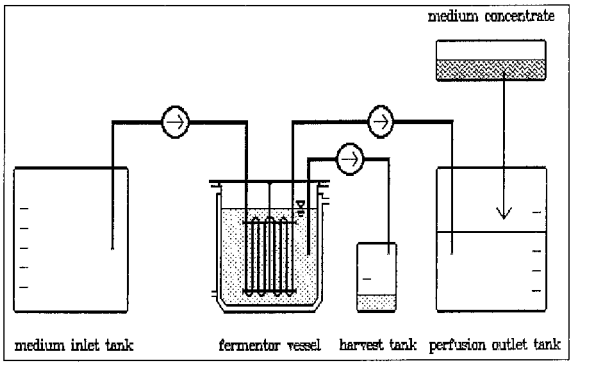

Fig. 2. MCB status at the end of the perfusion period (before supplementation).

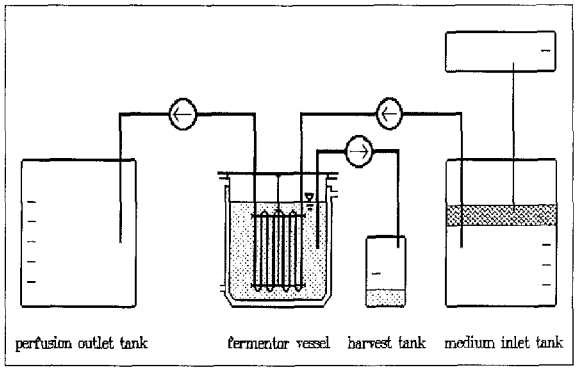

Fig. 3. MCB status at the start of the first recycling period (after supplementation).

contrary we pump the entire perfusion outlet medium back into the bioreactor and use it again. When using the double membrane stirrer, all you need to do is to change the direction of the perfusion pumps (Figs. 2 and 3) and to add a 'medium concentrate' in the same quantity as spent medium leaves the system via bleed. Hence, 80% of the spent medium is recycled. The product is taken out continuously by the bleed. The bleed rate, about 20% of the dilution rate, is performed as well to maintain a steady state of the culture and to avoid the accumulation of toxic substances by dilution (Kempken et al., 1990b). Only 20% instead of 100% of new medium is needed for the following perfusion period, which is equivalent to the first recycling period. This new medium is the 'medium concentrate', consisting of new medium supplemented with amino acids and glucose corresponding to the recycled volume (Table 1). The price for this concentrate is about the same as for new medium, because amino acids and glucose are very cheap in respect to the protein components (Table 2). Approximately the same nutrient composition and concentrations as in new medium are reinstalled, when the medium concentrate is added to the spent perfusion medium (Figs. 4, 5 and 6).

The first recycling period is done with the supplemented spent medium until the medium inlet tank is empty again. A new 20% of the dilution rate is leaving the system via bleed tank and 80% is collected cell-free in the perfusion outlet tank (Fig. 7). The bleed leaves the system for product harvest and is replaced as in the first recycling period by a volume of medium concentrate corresponding to the bleed volume (Fig. 8).

Table 1. Composition of medium and medium concentrate.

amino acid	A	B	C		Medium composition	
Asp	50	100	450	µM	1:1 mixture of DMEM and Ham's F 12	
Glu	50	150	700	µM	(basic medium), supplemented by:	
Asn	50	150	700	µM	50 µM	ethanolamine
Ser	250	500	2250	µM	2 mM	sodium pyruvate
His	150	300	1350	µM	500 mg/l	bovine serum albumin
Gln	2500	6500	30000	µM	10 mg/l	bovine insulin
Gly	250	500	2250	µM	10 mg/l	human transferrin
Thr	450	1350	6300	µM	besides	amino acids (B-A)
Arg	700	1400	6300	µM		
Ala	50	50	250	µM	List of amino acids (on the left side)	
Tyr	215	430	1935	µM		
Trp	45	90	405	µM	A: concentration in basic medium	
Met	115	345	1610	µM	(1:1 mixture of DMEM and F 12)	
Val	450	900	4050	µM	B: concentration in the supplemented	
Phe	215	430	1935	µM	basic medium (totally)	
Ile	415	830	3735	µM	C: concentration in medium concentrate	
Leu	450	1350	6300	µM	1 liter of it is used to supplement	
Lys	500	1000	4500	µM	4 liters of spent medium	
					C = 4·B + (B – A)	

Table 2. Costs for medium and medium concentrate.

Substance	Medium	Medium concentrate
bovine serum albumine	0.17 DM	0 DM
oleic acid	0 DM	0 DM
bovine insulin	6.20 DM	6.20 DM
human transferrin	2.58 DM	2.58 DM
ferric chloride	0 DM	0 DM
basic medium	1.40 DM	1.40 DM
sodium hydrogen carbonate	0.089 DM	0.089 DM
ethanolamine	0 DM	0 DM
sodium pyruvate	0.12 DM	0.12 DM
glutamine	0.20 DM	0.74 DM
glucose	0 DM	0.14 DM
extra pure water	0.16 DM	0.16 DM
amino acids (besides glutamine):	100 Pfg. = 1 DM (German Mark)	
Asp	0.085 Pfg.	0.43 Pfg.
Glu	0.088 Pfg.	0.44 Pfg.
Asn	1.80 Pfg.	9.01 Pfg.
Ser	3.20 Pfg.	15.98 Pfg.
His	1.80 Pfg.	8.99 Pfg.
Gly	0.11 Pfg.	0.55 Pfg.
Thr	5.71 Pfg.	28.57 Pfg.
Arg	2.60 Pfg.	12.99 Pfg.
Tyr	1.34 Pfg.	6.70 Pfg.
Trp	0.68 Pfg.	3.40 Pfg.
Met	0.96 Pfg.	4.80 Pfg.
Val	1.65 Pfg.	8.23 Pfg.
Phe	1.03 Pfg.	5.15 Pfg.
Ile	5.12 Pfg.	25.60 Pfg.
Leu	2.83 Pfg.	14.17 Pfg.
Lys	0.88 Pfg.	4.39 Pfg.
addition:	11.22 DM per liter	12.93 DM per liter

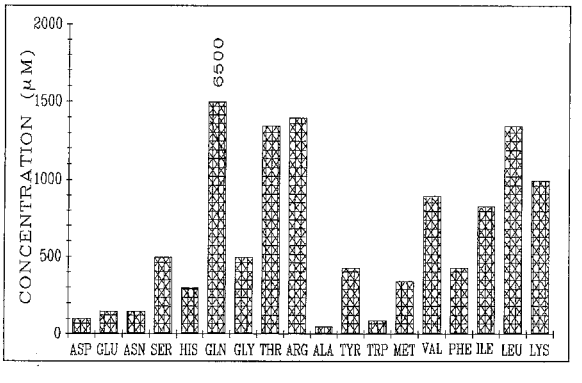

Fig. 4. Amino acid concentration pattern in the perfusion inlet tank of Fig. 1.

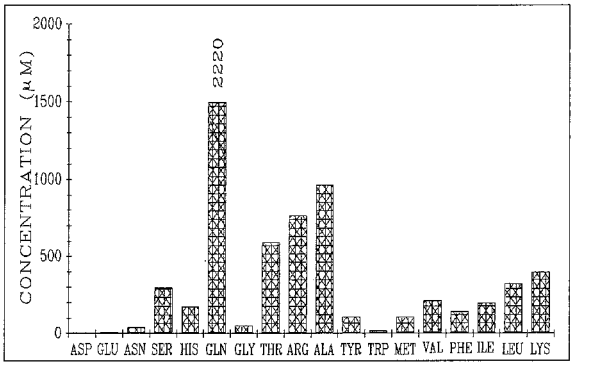

Fig. 5. Amino acid concentration pattern in the perfusion outlet tank of Fig. 2.

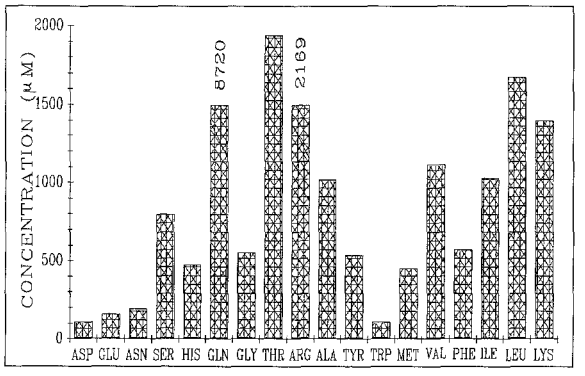

Fig. 6. Amino acid concentration pattern in the perfusion inlet tank of Fig. 3.

Again the concentrations and composition of nutrients are about equivalent to those in fresh medium (Figs. 9 and 10). In this way the second recycling period is started. The next periods of recycling and supplementation are alike.

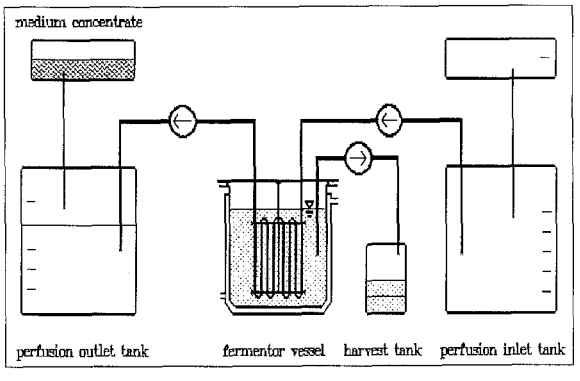

Fig. 7. MCB status at the end of the first recycling period (before supplementation).

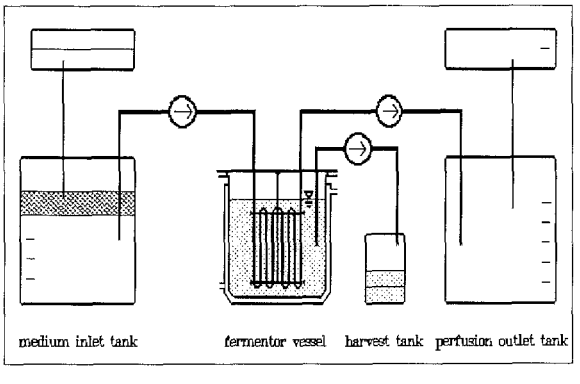

Fig. 8. MCB status at the start of the second recycling period (after supplementation).

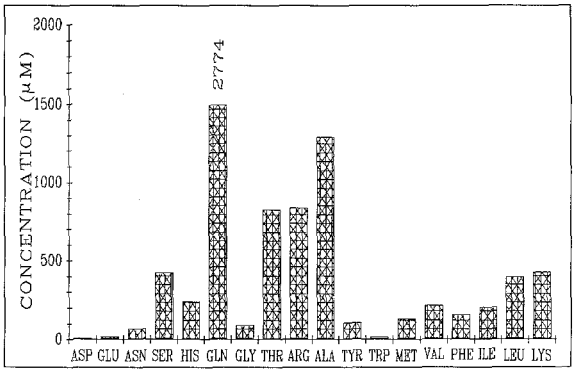

Fig. 9. Amino acid concentration pattern in the perfusion outlet tank of Fig. 7.

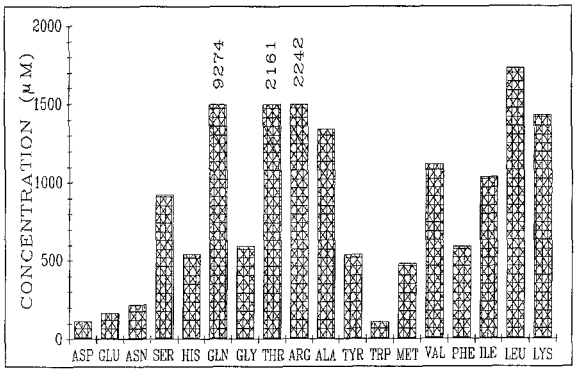

Fig. 10. Amino acid concentration pattern in the perfusion inlet tank of Fig. 8.

Protein content during a MCB fermentation

The protein content in the system is kept at a high level. Eighty percent of the high molecular weight substances are retained in a very simple way without using a technical retention unit. The process is uncomplicated and the costs for the retention unit are omitted. Conditioning factors produced by the cells persist in the MCB system. The protein components of the medium are likewise kept in the system and are not renewed. They mostly have catalytic or stimulating functions and are the main part of medium costs. Therefore, its retention in the system is a substantial benefit.

Product content during a MCB fermentation

The residence time of the product in the system is

enlarged considerably by the MCB procedure. If it is not absorbed, the product stays about five times longer in the MCB than in normal perfusion systems. In that time the product must be stable. Concerning the undermentioned culture the cell specific productivity was constant during continuous cultivation. Activity, stability and purity of the product have to be analyzed as well.

Inhibitors during a MCB fermentation

With a prolonged residence time it is important whether detrimental substances, formed and released by the cells, will accumulate beyond a tolerance level. The bleed rate has to be sufficient for the removal of inhibitors or toxic substances. Otherwise a loss in cell growth or productivity can be expected. Selecting an appropriate cell bleed rate the MCB can be used either to accumulate and detect toxic substances or to dilute those substances as a detoxification process.

Detoxification is by dilution only. Special elimination procedures for detrimental substances like lactate (Kempken et al., 1990a) or ammonium (Lütkemeyer et al., 1991, Kempken et al., 1991b, Newland et al., 1990) are omitted. Hence, the overall effect of inhibitors and toxic substances will arise during the MCB operation in the case when production reaches effective concentrations (Kempken et al., 1991c).

Nutrient requirements during a MCB fermentation

In contrast to other continuous culture processes, supplementation is restricted to those low molecular weight nutrients that are in fact consumed by the cells. In addition the supply of fresh medium is necessary to equate with the spent medium that leaves the system via bleed (Kempken et al., 1990b). Hence, many medium components have to be analyzed, e.g. glucose, amino acids, organic acids, inorganic ions. A suitable supplementation mixture is calculated from the analysis data and serves as a standardized medium concentrate for that particular cell line.

Savings due to the MCB process

During the recycling run about 80% of medium and therefore costs are saved. Since the requirement for new media is reduced, time and costs for medium preparation, sterile filtration and sterile testing are also saved. We also get a drop in costs for inactivation of spent medium if it is demanded by law (Hasskarl, 1990a) and preferably performed by autoclaving (Hasskarl, 1990b) with high energy costs.

The entire MCB system is operated sterile. Additional sterile filtration procedures due to the recirculation process are omitted. The MCB process is cheap and uncomplicated because proteins are re-used without a technical retention system and because detoxification is by dilution only. We expect a drop of process costs especially in large scale production systems.

Materials and methods

The basic bioreactor was a B. Braun Biostat BF (B. Braun Diessel Biotech GmbH, FRG) at a two liter scale. It was equipped with a double membrane stirrer with 5.30 m polypropylene hollow fiber membrane tubing for aeration and 3.30 m hydrophilized polypropylene hollow fiber membrane tubing for cell-free medium exchange. A bioreactor control unit DCU (B. Braun Diessel Biotech GmbH, FRG) and a perfusion mode with medium backflush procedure as described by Büntemeyer (1988) were used. The microfiltration membrane (Enka AG, FRG) was connected at the input side via a peristaltic pump (Watson-Marlow Ltd., GB) with the medium inlet tank and at the output side via another pump with the perfusion outlet tank. These two perfusion pumps were controlled to create a process cycle of alternatively harvesting and feeding depending on reactor volume and pump speed. Temperature was set at 37°C, stirrer speed to 30 rpm, pH to 7.10 and gas supply to 40% air saturation.

The cell line used in this study was a mouse-rat hybridoma (ATCC HB 58). This cell line secretes a rat monoclonal antibody IgG_1 type specific for

mouse antibody κ light chains. Two or three samples per day were taken from the bioreactor vessel and analyzed as follows. The cell number was counted microscopically by trypan blue exclusion. Glucose and lactate were analyzed using the YSI 2000 analyzer (Yellow Springs Instruments, Ohio, USA), ammonium using an ammonia selective electrode with microprocessor pH/ION meter pMX 2000 (WTW GmbH, FRG). The free amino acids in the samples were analyzed by the automated reversed phase HPLC system 400 (Kontron GmbH, FRG) with precolumn derivatization using the OPA method (Büntemeyer et al., 1991). Antibody concentrations in the supernatant were analyzed using a kinetic sandwich ELISA method and the EL 311 autoreader (Tecnomara GmbH, FRG).

The serum-free medium used in this study was a 1:1 mixture of DMEM and Ham's F 12, supplemented by 50 µM ethanolamine, 2 mM sodium pyruvate, 500 mg/l BSA, 10 mg/l human transferrin and 10 mg/l bovine insulin. In addition the following amino acid concentrations were added: Asp 50 µM, Glu 100 µM, Asn 100 µM, Ser 250 µM, His 150 µM, Gln 4000 µM, Gly 250 µM, Thr 900 µM, Arg 700 µM, Tyr 215 µM, Trp 45 µM, Met 230 µM, Val 450 µM, Phe 215 µM, Ile 415 µM, Leu 900 µM, Lys 500 µM.

This medium was used for batch cultivation and for perfusion until medium recycling started. At the start of the recycling periods (at 90 h and 137 h culture time) the bleed, leaving the system, was replaced by medium concentrate of the same quantity. Medium and medium concentrate compositions are listed in Table 1, its costs in Table 2.

The culture was divided into seven periods (Table 3) to calculate the specific productivity. Periods 4 and 6 were formed, as the cell bleed started at the end of the first perfusion period and as the bleed rate was raised at the end of the first recycling period.

Functions were correlated mathematically to the growth curve (Fig. 11) and to the product concentration curve (Fig. 12). From the function, the first order derivative of the function and the equations for the cell specific parameters (Pirt, 1985), the specific productivities were calculated for the system and for its sections (bioreactor vessel, perfusion loop, bleed tank).

Results

The growth curve is shown in Fig. 11. Using an inoculum from another bioreactor, cell proliferation and product formation started at relatively high concentrations and without a lag period. During batch phase growth was unlimited (Figs.

Table 3. Periods of the MCB fermentation.

period 1	batch
(batch phase)	t = 0 h to 25.75 h
period 2	perfusion with Dp = 0.62/d
(1st perfusion phase)	t = 25.75 h to 49 h
period 3	perfusion with Dp = 0.95/d
(1st perfusion phase)	t = 49 h to 74.5 h
period 4	perfusion with Dp = 0.95/d
(1st perfusion phase)	bleed with Db = 0.23/d
	t = 74.5 h to 90 h
period 5	perfusion with Dp = 0.95/d
(1st recycling phase,	bleed with Db = 0.23/d
2nd perfusion phase)	t = 90 h to 123.25 h
period 6	perfusion with Dp = 0.95/d
(1st recycling phase,	bleed with Db = 0.52/d
2nd perfusion phase)	t = 123.25 h to 137 h
period 7	perfusion with Dp = 0.95/d
(2nd recycling phase,	bleed with Db = 0.52/d
3rd perfusion phase)	t = 137 h to 175 h

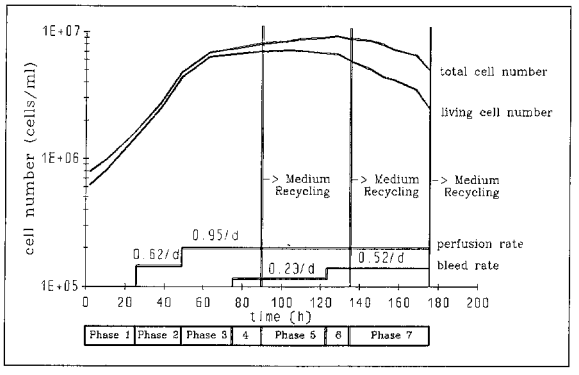

Fig. 11. Growth curve.

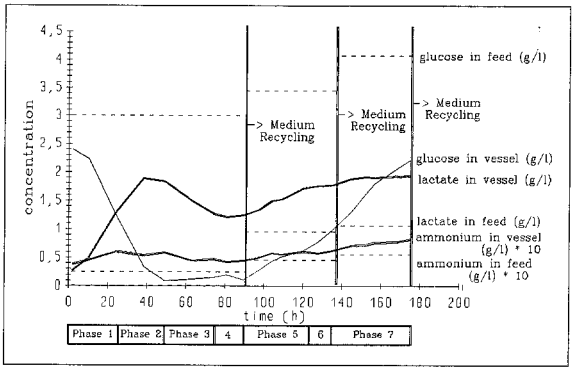

Fig. 13. Concentrations of glucose, lactate, ammonium.

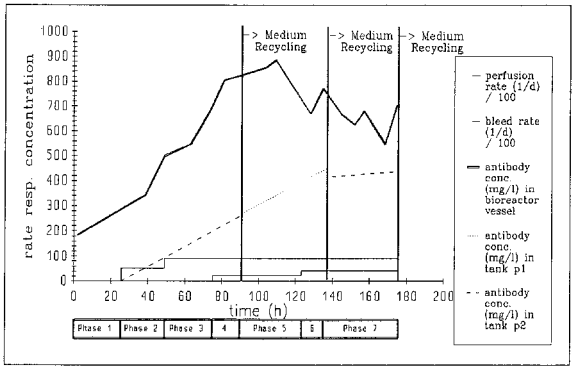

Fig. 12. Product concentrations.

11 and 13). A growth rate of 1.05 per day was stable up to the start of period 3. Then growth was limited by numerous components (Fig. 13), changed into a steady state at 7 million viable cells per ml and remained constant during the recycling (period 5). At period 6 the bleed rate was raised distinctly in respect to the accumulation of dead cells. Subsequently a decrease of viable cells was observed in the bioreactor.

Glucose, lactate and ammonium concentrations are plotted in Fig. 13. The glucose uptake rate in batch phase was 1.20 g per day per 10^9 cells. The production rates for lactate, ammonium and alanine in batch phase were 0.93 g, 17.2 mg and 35.0 µM per day per 10^9 cells. During the first perfusion period glucose was totally consumed. Subsequently the glucose concentration was raised to nearly double that in the intake by increased supplementation via feed. The course of amino acid metabolism looked similar, uptake rates are listed in Table 4, and concentrations at turning-points of the periods in the presented culture are shown in Figs. 4, 5, 6, 9 and 10. The concentrations of the potential inhibitors lactate, ammonium and alanine kept constant during the continuous cultivation. Neither lactate (≤2.0 g/l) nor ammonium (≤82 mg/l) reached a level that is

Table 4. Cell specific uptake rates during the batch period of the MCB fermentation.

Amino acid	Uptake rate (µmol/d·10^9 cells)
Asp	42.2
Glu	21.6
Asn	29.8
Ser	70.7
His	91.0
Gln	3650
Gly	18.3
Thr	183.6
Arg	184.0
Tyr	135.8
Trp	25.8
Met	72.8
Val	183.5
Phe	78.8
Ile	180.0
Leu	291.0
Lys	259.0
Ala	was produced
Cys	was not analyzed
Pro	was not analyzed
Glucose	1.20 g/d·10^9 cells

toxic in our experience, and probably the same is valid for alanine (≤2 mM).

Figure 12 illustrates the product concentration in the bioreactor vessel and in the perfusion tanks p1 and p2. The product titer in the bioreactor increased to 850 mg/l during the perfusion mode (period 2 to 4). At the same time about 260 mg/l were collected in the perfusion outlet tank (see data 'antibody concentration in tank p2' of Fig. 12). At period 4 the dilution rate was increased by activating the bleed. In the sequel the product concentration in the bioreactor was between 600 to 700 mg/l. At the same time the perfused medium with 260 mg/l product was re-used. In the course of the first recycling period (period 5 and 6) the product concentration rose to 450 mg/l (see data 'antibody concentration in tank p1'). In period 6 the bleed rate was distinctly increased. During the second recycling period (period 7) the product concentrations remained constant in the bioreactor and in the perfusion tanks (Fig. 12). Further product was only gained in the bleed.

Calculation of the cell specific parameters show a constant cell specific productivity during the fermentation (Table 5). Therefore, the medium recycling had no detrimental effect on product formation. The volumetric productivity decreased during the second recycling period. This is certainly not due to the recycling operation but due to the decrease in viable cell number at this period (see Table 5 and Fig. 11). The latter obviously dropped because of the increased bleed rate (0.52/d). Apparently a growth inhibition occurred, because μmax was 1.05/d in batch phase and the analyzed substrates (glucose, amino acids besides cysteine and proline) were no longer minimal at that time.

The overall product concentration in the entire system (bioreactor vessel, bleed tank, perfusion tanks) is shown in Fig. 14. At period 1 to 4 (Batch and perfusion with fresh medium) 2997 mg antibody were produced in 90 h. At period 5 to 6 (first recycling) 1349 mg were formed in 47 h and at period 7 (second recycling) 681 mg were formed in 38 h (Fig. 14). The production efficiency in advance of the recycling (33 mg/h) did not change substantially during the first recycling (29 mg/l) and decreased during the second recycling (18 mg/l).

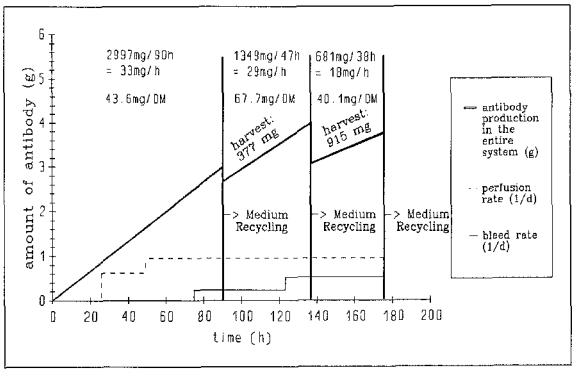

Fig. 14. Product concentration in the entire system.

The medium costs are also mentioned in Fig. 14. For a medium investment of one German Mark, 43.6 mg antibody was produced in advance of the recycling, 67.7 mg were produced during the first recycling and 40.1 mg during the second recycling. Referred to the expended medium costs the 1.5 fold product quantity was obtained in the first and the same quantity was obtained in the second recycling period as in the perfusion period with fresh medium.

Using the recirculated medium instead of fresh medium, the medium costs were reduced from 56.10 DM to 12.93 DM in respect of the first recycling (saving of 77%), and from 56.10 DM to 16.81 DM in respect to the second recycling (saving of 70%). Applied to the overall culture

Table 5. Specific (q) and volumetric (Q) productivities during the seven periods of the MCB fermentation.

Period	1	2	3	4	5	6	7
q (mg/d·10^9 cells)	121	127	113	147	144	not calculated	144
Q (mg/d·l)	103	375	690	1024	1040	not calculated	630

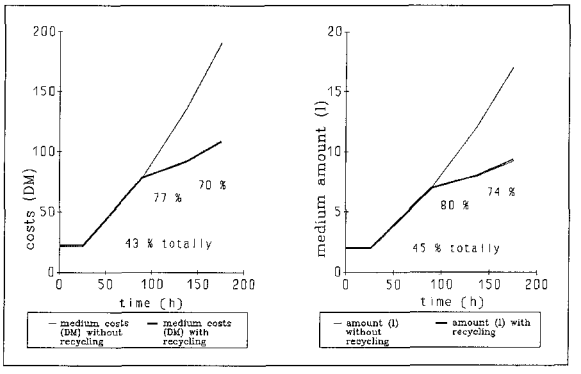

Fig. 15. Economy of the MCB. Medium costs (left), medium amount (right).

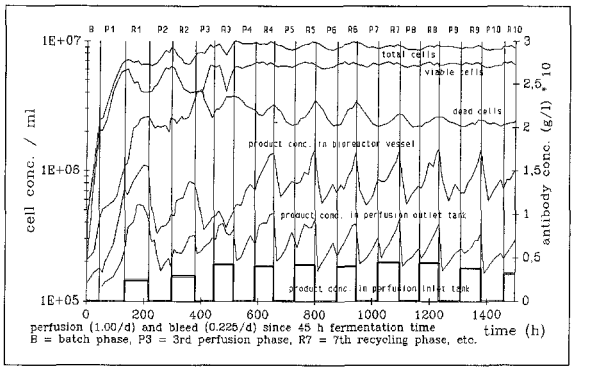

Fig. 16. Growth and product concentration curves of a long-term MCB fermentation.

43% of the costs for the serum-free medium were saved (Fig. 15).

The reduction of waste water quantity, which has to be inactivated before disposal, was 80%, 74% and 45% regarding the first, second recycling step and the overall culture respectively (Fig. 15).

A one-fold recirculation of the spent medium is recommended for this culture. For the inspected cell line the specific and volumetric productivity, in addition to the cell growth during the first recycling period, were comparable to those at the preceding perfusion period with fresh medium. During the second recycling period the volumetric productivity decreased. This can be explained by the decreasing number of viable cells in the bioreactor or to a degradation of the product. Product degeneration should be avoided by cooling the peripheral medium tanks. The cell decrease was caused by too great an increase in bleed rate at that time, or by growth inhibition as a result of insufficient detoxification of detrimental substances in the medium. If medium recycling and medium exchange are done alternately, the inspected culture should be able to be performed also in long-term operation.

Consequently, the medium cycle bioreactor concept was tested in long-term operation in the same bioreactor unit in 2 liter scale. In this subsequent study the spent perfusion medium was re-used once (as recommended above) and then discarded and replaced by fresh medium that was also re-used once and then discarded, etc.

Figure 16 shows the result of a 1500 h fermentation (62.5 days). As in the previous fermentation (Figs. 11–13) the vertical bars in Fig. 16 indicate the start of respective new perfusion and recycling periods. Beyond the 45 h batch period constant rates for perfusion (1.00/d) and cell bleed (0.225/d) were run. P1 is the first perfusion period with the fresh medium, followed by medium re-use, supplementation and by the first recycling period (R1), then followed by use of new medium and by the second perfusion period (P2), then followed by medium re-use, supplementation and by the second recycling period (R2), then followed by P3, R3, P4, R4, P5, etc.

The cells grew to nearly 7 million viable cells per ml at the end of P1. Interruption of nutrient feed or gas supply caused a decline in cell number during R1, R2 and R3, but the cells recovered well each time. Since P4 no further complications arose and a steady state at constant cell number was maintained. Thus, the cell growth was not affected perceptibly by the numerous one-fold recycling procedures.

Below the uppermost three curves (total, viable and dead cells) of Fig. 16 the antibody concentrations in the bioreactor vessel (upper twisted curve), in the perfusion outlet tank (lower twisted curve) and in the perfusion inlet tank during the R periods (intense beam curve) are plotted. Obviously, the antibody concentrations during the P periods (fresh medium) were zero in the perfusion inlet tank. Antibody production was about the same both during perfusion periods with fresh

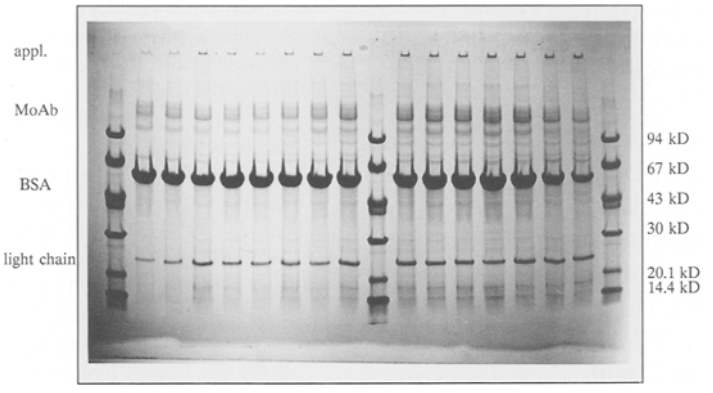

Fig. 17. SDS-PAGE of bioreactor samples from different P or R periods. Indicated at the left: samples (lane 2–9 and 11–17): non-reduced, appl.: sample application area, MoAb: monoclonal antibody (121 kD), BSA: bovine serum albumin (54 kD), light chain: additional light chain of monoclonal antibody (23 kD). Indicated at the right: standard (lane 1, 10, 18): reduced, phosphorylase b (94 kD), albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20.1 kD), lactalbumin (14.4 kD).

and re-used medium respectively and during the different culture periods. Thus, medium recycling is suitable for long-term application. To indicate the stability of the product, a gel electrophoresis was made of bioreactor samples in the middle of different P or R periods (Fig. 17). The antibody zones were consistent during the culture. With reference to the overall culture with one-fold medium re-use, the medium costs were reduced from 1835 DM to 1150 DM (saving of 37%) and the medium volume was reduced from 161 liter to 100.5 liter (saving of 38%).

Discussion

Medium recycling is feasible in a very simple way, as shown in the presented culture. Setting suitable flow rates for perfusion and bleed leads to a renunciation of technical systems for protein retention and inhibitor removal. The recycling procedure achieves considerable reduction of medium costs and a substantial drop of waste water quantity. The medium cycle bioreactor uses the classic perfusion mode and needs about the same expense for construction and operation as classic perfusion systems. The MCB permits a homogeneous cultivation in steady state with extremely economic exploitation of the cell culture medium.

How many times may the spent medium be recirculated? In general the recycling procedure can be repeated until harmful effects occur for cell growth or productivity. If those effects appear, the recycled medium should be taken out of the system and be replaced by fresh medium. After that the recycling procedure starts anew.

Subsequent studies will investigate long-term operation with this system. Besides, possible inhibitors ought to be detected quickly with a variant of the MCB. With this, the cell-free perfusion outlet fluid is collected directly in the perfusion inlet tank again. Simultaneous to the cell bleed the same quantity of medium concentrate is pumped constantly into the perfusion inlet tank. In this especially dynamic recirculation system, detrimental events would take effect at once to the cell culture.

Such effects relative to the specific productivity were not obtained during the second recycling phase of the presented 175 h culture. Maintaining the primary bleed rate probably did not diminish the viable cell number and hence the volumetric productivity during the second recycling period. Finally, the 1500 h culture with one-fold medium recycling and constant bleed rate (20% of the total dilution rate) gave no indication that cell growth or specific and volumetric productivity deteriorated when the spent and supplemented medium was fed into the bioreactor. Similar to

our results, Mizrahi and Avihoo (1977) found that the spent medium could be re-used once and that loss in cell growth appeared during a two-fold re-use.

Apparently this MCB process with one-fold medium re-use can be used long-term and is as elegant and as effective as a normal perfusion system. Although the medium recycling and with this the economic period only occurred for every other phase (R1, R2, R3, etc.), more than a third of cell culture water (38%) and medium costs (37%) were saved. Hence, with a one-fold recycling we already get a substantial reduction of process costs.

Acknowledgement

The financial support by the B. Braun Diessel Biotech GmbH and by the project 'Development of a procedure and a plant for the recirculation of nutrient media for animal cell culture' (BMFT project reference no. 0319346A) of the German Federal Ministry of Research and Technology is acknowledged.

References

Büntemeyer H, Bödeker BGD and Lehmann J (1987) Membrane-stirrer-reactor for Bubble Free Aeration and Perfusion. In: Modern Approaches to Animal Cell Technology, Spier RE and Griffiths JB (eds), Butterworth, London, 411–419.

Büntemeyer H (1988) PhD Thesis, University of Hannover, FRG.

Büntemeyer H, Bödeker BGD and Lehmann J (1990) Ein Zellkulturfermenter mit integriertem Membransystem für die homogene Kulturführung. Chem. -Ing. -Tech. 62, No. 5, 393–395.

Büntemeyer H, Lütkemeyer D and Lehmann J (1991) Optimization of Serum-free Fermentation Processes for Antibody Production. Cytotechnology 5: 57–67.

Chmiel H and Strathmann H (1985) Membranen in der Verfahrenstechnik. Chem. -Ing. -Tech. 57, No. 7, 581–596.

Hasskarl H (1990a) Gentechnikrecht: Textsammlung (Gentechnikgesetz und Rechtsverordnungen). Editio Cantor Verlag, Aulendorf.

Hasskarl H (1990b) ditto, p. 110, p. 148, p. 154.

Kempken R, Büntemeyer H and Lehmann J (1990a) Cell Culture Medium Detoxification by Electrodialysis. Poster No. 11 at 12th July, 5th European Congress on Biotechnology, Copenhagen, 9th–13th July 1990.

Kempken R, Büntemeyer H and Lehmann J (1990b) The Medium Cycle Fermentor: a New Approach for Medium Recycling and Reduction of Fermentation Process Costs. Proceedings of the German-Japanese Workshop on Animal Cell Culture Technology, Hamburg-Harburg, 5th–6th November 1990, in press.

Kempken R, Büntemeyer H and Lehmann J (1991a) Medium-Kreislauf-Fermenter minimiert Kosten und Abwasser. Poster No. 161, 9. DECHEMA Jahrestagung der Biotechnologen, 0st-Berlin, 30.01.–01.02.

Kempken R, Lütkemeyer D, Heidemann R, Maurer U, Büntemeyer H and Lehmann J (1991b) Elimination of Ammonium and Lactate as Toxic Agents in Animal Cell Cultures. Poster at the 4th Tutzing Symposium on Chemical Engineering in Medicine and Biotechnology, Tutzing (FRG), 25th–28th February 1991.

Kempken R, Büntemeyer H and Lehmann J (1991c) Biological and Technological Aspects of Medium Recycling with the Medium Cycle Fermentor (MCF). Lecture at the 4th Tutzing Symposium on Chemical Engineering in Medicine and Biotechnology, Tutzing (FRG), 25th–28th February 1991.

Lehmann J, Vorlop J and Büntemeyer H (1988) Bubble-free Reactors and Their Development for Continuous Culture with Cell Recycle. In: Animal Cell Biotechnology, Spier RE and Griffiths JB (eds), Academic Press, London, Vol. 3, 221–237.

Lehmann J, Kempken R, Lütkemeyer D and Büntemeyer H (1989) Economic Aspects of Medium Recycling. Trends in Animal Cell Culture Technology (Proceedings of the 2nd Annual Meeting of the JAACT, Tsukuba, Japan, 20th–22th November 1989), Murakami H (ed), VCH Publishers 1990, 55–59.

Lütkemeyer D, Heidemann R, Maurer U, Büntemeyer H and Lehmann J (1991) Mediumrezyklisierung mit Ammoniumentgiftung im 201 Perfusionsfermenter. Poster No. 68, 9. DECHEMA Jahrestagung der Biotechnologen, 0st-Berlin, 30.01–01.02.

Mizrahi A and Avihoo A (1977) Growth Medium Utilization and its Re-use for Animal Cell Cultures. Journal of Biological Standardization 5: 31–37.

Newland M, Greenfield PF and Reid S (1990) Hybridoma Growth Limitations: The Roles of Energy Metabolism and Ammonia Production. Cytotechnology 3: 215–229.

Pirt SJ (1985) Principles of Microbe and Cell Cultivation, Blackwell Scientific Publications pp. 209–222.

Rønning OW and Schartum M (1990) Removal of Inhibitory Factors from Hybridoma Cell Cultures by Gel Filtration. Production of Biologicals from Animal Cells in Culture (Proceedings of the 10th ESACT Meeting, Avignon, 7th–11th May 1990), Spier RE, Griffiths JB and Meignier B (eds), Butterworth-Heinemann Ltd. 1991, 218–223.