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# Datasheet for the decision of 22 August 2024

**Case Number:** T 0880/21 - 3.3.08

**Application Number:** 08797478.8

**Publication Number:** 2188371

**IPC:** C12P21/00, C12M1/00, C12N5/00

**Language of the proceedings:** EN

**Title of invention:**
Use of perfusion to enhance production of fed-batch cell culture in bioreactors

**Patent Proprietor:**
Wyeth LLC

**Opponents:**
F.Hoffmann-La Roche AG
European Oppositions Limited
Glaxo Group Limited
Merck Sharp & Dohme LLC

**Headword:**
Combined perfusion-fed-batch process/WYETH

**Relevant legal provisions:**
EPC Art. 56

**Keyword:**
Main request and auxiliary requests 1 to 3 - inventive step - (no)

**Decisions cited:**
T 0892/08, T 1179/16, T 0261/19

**Catchword:**

Europäisches Patentamt
European Patent Office
Office européen des brevets

**Beschwerdekammern**

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**Case Number: T 0880/21 - 3.3.08**

# D E C I S I O N
# of Technical Board of Appeal 3.3.08
# of 22 August 2024

| | |
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| **Decision under appeal:** | **Decision of the Opposition Division of the European Patent Office posted on 20 April 2021 rejecting the opposition filed against European patent No. 2188371 pursuant to Article 101(2) EPC** |

**Composition of the Board:**

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| **Chair** | T. Sommerfeld |
| **Members:** | M. Montrone |
| | D. Rogers |

## Summary of Facts and Submissions

I. Appeals were lodged by opponents 1 to 4 ("appellants I to IV", respectively) against the decision of an opposition division to reject the oppositions against European patent No. 2 188 371. This patent is based on European patent application No. 08 797 478.8 which was filed as International patent application published as WO 2009/023562.

II. The opposition proceedings were based on all grounds under Article 100(a) to (c) EPC.

III. In their statements of grounds of appeal ("SGA"), appellants I to IV *inter alia* argued that the subject-matter of the claims as granted lacked an inventive step (Article 100(a) EPC in relation to Article 56 EPC).

IV. In reply, the patent proprietor ("respondent") provided counter arguments and re-submitted auxiliary requests 1 to 7 already filed during the opposition proceedings in reply to the opponents' notices of opposition.

V. Appellants III and IV filed further submissions in response to the respondent's reply to the appeals.

VI. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's preliminary opinion.

VII. Appellant I filed further arguments in reply to the board's communication. With letter dated 29 July 2024, the respondent withdrew their main request (claims as granted) and auxiliary requests 1 to 3 filed in reply

to the appeals. Furthermore auxiliary request 4 filed in reply to the appeals became the new main request while auxiliary requests 5 to 7 filed with the reply too were renumbered as auxiliary requests 1 to 3. All maintained requests were re-submitted with the new numbering.

VIII. Oral proceedings were held in the presence of all parties.

IX. Claim 1 of the main request reads:

"1. A cell culture method for production of a polypeptide comprising the steps of:

(a) growing cells in a cell culture to a first critical level wherein said first critical level is reached at

- a cell density of 1 million to 9 millions cells per milliliter,

- a lactate concentration level of 1 g/L to 6g/L or,

- at day 1 to day 5 of the cell culture;

(b) perfusing the cell culture, wherein perfusing comprises replacing spent medium with fresh medium, whereby at least some portion of the cells are retained and at least one waste product is removed;

(c) growing cells in the cell culture to a second critical level wherein said second critical level is reached at

- a cell density of 5 million to 40 million cells per milliliter, or,

- at day 2 to day 7 of the cell culture;

(d) initiating a polypeptide production phase by a change in temperature, pH or osmolality of the cell culture or combination thereof; and

(e) maintaining cells in a fed-batch culture during at least some portion of the polypeptide production phase,

wherein the cell culture is a CHO cell culture and,

wherein the polypeptide produced by the cell culture is an antibody".

X. Claim 1 of auxiliary request 1 differs from that of the main request in that in step (c) the feature "*a cell density of 5 million to 40 million cells per milliliter*" has been deleted.

XI. Claim 1 of auxiliary request 2 differs from that of auxiliary request 1 in that in step (d) the feature "*wherein the step of initiating the polypeptide production phase comprises a temperature shift in the cell culture*" has been added.

XII. Claim 1 of auxiliary request 3 differs from that of auxiliary request 2 in that the feature "*wherein the at least one waste product is removed by passing the spent medium through an ultrafiltration device*" has been added at the end of the claim.

XIII. The following documents are referred to in this decision:

D1: Müller D. *et al.*, Animal Cell Technology: From

Target to Market, 2001, Kluwer Academic Publishers, 293-299

D6: Yoon S. K. *et al.*, Applied Microbiology and Biotechnology, 2007, Vol. 76, 83-89

D12: Excerpt of Cell Culture Technology for Pharmaceutical and Cell-Based Therapies, 2005, Eds. S.S. Ozturk and W.-S. Hu; Chapter 10: Xie L. and Zhou W., Fed-Batch Cultivation of Mammalian Cells for the Production of Recombinant Proteins, 349-386

D13: Excerpt from Cell Culture Technology for Pharmaceutical and Cell-Based Therapies, 2005, Eds. S.S. Ozturk and W.-S. Hu; Chapter 11: Kompala D.S. and Ozturk S.S., Optimization of high cell density perfusion bioreactors, 387-416

D18: Chen Z.-E. *et al.*, Journal of Bioscience and Bioengineering, 2004, Vol. 97(4), 239-243

D72: Declaration of Gregory Hiller, dated 9 April 2019

D89: Declaration of Gaurav Chauhan, dated 27 August 2021

XIV. The appellants' submissions, insofar as relevant to the present decision, may be summarised as follows:

*Main request*

*Claim construction -claim 1*

The method as defined in claim 1 did not specify that a particular amount of antibodies was produced. Step (d)

of claim 1 defined a qualitative effect (i.e. that protein production was initiated) but no quantitative effect since claim 1 did not require that after triggering the production phase the majority of the antibody was produced. This was so because claim 1 encompassed short production phases due to the absence of any time specification. Nor did step (d) require that perfusion culturing was terminated after initiating the production phase. In fact the duration of perfusion culturing was left open by claim 1 as supported by the subject-matter of dependent claim 11.

*Inventive step - claim 1*

Document D1 was a suitable closest prior art. The claimed method differed from the method disclosed in document D1 by the features of step (d) and in that an antibody was produced. There were no comparative data on file which disclosed or rendered credible an advantageous effect of these distinguishing features when compared to the method of document D1. The working examples of the patent, for example, lacked proper controls for assessing potential effects of the production initiation as defined in step (d) on yield and culture duration. Nor were any technical effects ascribable to the production of an antibody when compared to the cytotoxic protein of document D1.
The technical problem to be solved by the method of claim 1 resided in the mere provision of an alternative cell culture method for recombinant protein production. The claimed method as solution to this problem was obvious for the skilled person starting from document D1 taking common general knowledge into account. The use of, for example, temperature shifts for triggering protein production in cell culturing belonged to the skilled person's common general knowledge (documents D6

and D18, for example) as well as the use of CHO cells for the production of antibodies (document D12). Nor did the patent provide data that the time point of triggering the production phase (i.e. after reaching the second critical level) as specified in step (d) of claim 1 was associated with any advantageous technical effect. On the contrary, the patent disclosed that this time point had no effect on yield, let alone on culture duration (Examples 2.2 to 2.4). Document D1 did also not teach away from combining perfusion culturing with fed-batch. On the contrary, the document consistently reported that the combination of both cultivation types resulted in the highest yields. Nor did the yield go down during fed batch culturing (document D89). Further the selection of antibodies was arbitrary since any protein could be produced by the claimed method. This feature had thus to be disregarded in the assessment of inventive step. The method of claim 1 was thus obvious for the skilled person starting from document D1 and taking common general knowledge into account.

*Auxiliary request 1*

*Inventive step - claim 1*

The specific duration of cultivation as defined in step (c) referred to an arbitrary number of days since this period was not linked to any parameter relevant for a cell culture-based protein production. This feature had thus to be disregarded for assessing inventive step.

*Auxiliary request 3*

*Inventive step - claim 1*

Comparative data between the use of an ultrafiltration ("UF") device and the ultrasound-based cell retention device disclosed in document D1 were not available for supporting potential advantageous effects of UF. Even if, as argued by the appellant, the cell retention properties of microfiltration ("MF") and ultrasound-based devices were comparable, Examples 2.3 and 2.4 of the patent did not demonstrate that the use of UF devices improved the yield and shortened the culture duration compared to MF devices. Moreover the use of UF devices in the production of antibodies belonged to the skilled person's common general knowledge (document D13, page 390, fourth paragraph). The method of claim 1 of auxiliary request 3 lacked thus an inventive step over the teaching of document D1 taking common general knowledge into account.

XV. The respondent's submissions, insofar as relevant to the present decision, may be summarised as follows:

*Main request*

*Claim construction - claim 1*

The initiation of protein production as defined in step (d) of claim 1 had the effect that the majority of antibody was produced (document D72). This was due to the commonly known metabolic shift in cells which favoured protein production over cell proliferation. The duration of perfusion culturing as specified in claim 1 was also not open ended. Applying a mind willing to understand, the skilled person did not elongate perfusion for an unreasonable period of time after triggering the production phase.

*Inventive step - claim 1*

Document D1 was not a suitable closest prior art since it related to a different purpose, i.e. the production of a cytotoxic protein instead of an antibody. Its selection as prior art relied on hindsight knowledge of the claimed method.
If document D1 was used as a springboard, the method of claim 1 differed therefrom by the features of step (d) and the production of antibodies. The features in step (d) specified the time point in the claimed method when the majority of the protein was produced. This was supported by Example 2.2 in conjunction with Figure 8 of the patent which disclosed that a temperature shift (as an embodiment of step (d) of claim 1) led to a high yield of antibodies within a short cultivation period. The yield disclosed in Figure 8 of the patent was at least 4-fold higher than that reported in document D1 (abstract). Furthermore, document D1 disclosed that the highest yield was obtained only after about two months of cell culturing (Figure 2) while it was significantly shorter in Figure 8 of the patent.
Document D1 was also silent on any initiation of protein production, let alone that this occurred after the cell culture had reached its second critical level as defined in step (c) of claim 1. In the absence of any pointer in document D1 to initiate protein production at this specific stage, the claimed method was inventive over the teaching of document D1.
Document D1 also taught away from combining perfusion culturing with fed-batch. Document D1 reported on losses of cell viability and increased cell lysis during fed-batch. Also the yield went down at the end of fed-batch culturing (document D89). Since this was detrimental for the protein produced, the skilled person rather avoided the combination of perfusion and fed-batch culturing for obtaining high antibody yields.

Instead the skilled person would optimise the conditions of perfusion culturing. Further the fact that a combined perfusion fed-batch culturing that relied on an initiation of the production phase had not been disclosed between the publication of document D1 and the filing date of the patent represented a secondary indication for the presence of an inventive step.

*Auxiliary request 1*

*Inventive step - claim 1*

The method of claim 1 was limited in that a specified duration of cell culturing indicated that the second critical level of perfusion culturing was reached. The perfusion culturing of the claimed method was therefore short when compared to document D1. This had the effect that costs were saved because less medium was needed.

*Auxiliary request 3*

*Inventive step - claim 1*

The retention properties of a MF device were comparable to the ultrasound device reported in document D1. Thus the experimental data in the patent (Figures 12 and 18), which compared the effects of UF devices and MF devices, were suitable in supporting advantageous effects on titre, yield and culture duration of the claimed UF devices versus the ultrasound device of document D1. Nothing in document D1 pointed the skilled person to the use of UF devices, let alone that thereby higher yields of antibodies at a shorter perfusion were obtainable.

XVI. The relevant requests of the parties for the decision are the following (for the complete list of the parties' requests, see the minutes of the oral proceedings):

(a) The appellants requested that the decision under appeal be set aside and the patent be revoked.

(b) The respondent requested that the patent be maintained on the basis of the claims of the main request or auxiliary requests 1 to 3 filed with letter dated 29 July 2024 and corresponding, respectively, to auxiliary requests 4 to 7 filed with the reply to the appeals.

## Reasons for the Decision

*Main request*

*Claim construction - claim 1*

1. Claim 1 is directed to a "*cell culture method for production of a polypeptide*" characterised by process steps (a) to (e), wherein "*a CHO cell culture*" is used and the polypeptide produced "*is an antibody*". The use of further process steps is not excluded from claim 1 due to the use of the "*comprising*" language.

2. The claimed cell culture method comprises three phases, two growth phases characterised by process steps (a) to (c) and an antibody production phase characterised by process steps (d) and (e).

3. The first growth phase is specified in step (a) wherein CHO cells are grown in culture until the culture has

reached a "*first critical level*". This first level is defined by three alternative parameters:

- a cell density ("*1 million to 9 millions cells per milliliter*"),
- a lactate concentration ("*1 g/L to 6g/L*"), or
- a cell culture duration ("*day 1 to day 5*").

In the absence of further specifications, step (a) requires that CHO cells are grown by any suitable method until a first critical level is reached, i.e. a cell culture stage indicated by one of the three alternative parameter ranges.

4. The second growth phase is specified in step (b) wherein the cell culture is perfused which requires "*replacing spent medium with fresh medium, whereby at least some portion of the cells are retained and at least one waste product is removed*".

4.1 Step (b) requires a cell culturing by perfusion which according to the skilled person's ordinary understanding relates to "*the continuous inflow of nutrient medium coupled with the outflow or harvesting of spent medium, while the cells are fully or partially retained within the bioreactor*" (see e.g. document D13, page 387, first paragraph). This type of cell culturing requires a simultaneous addition of fresh medium to the culture and a removal of spent medium from the culture while cells at least in part are retained in the reactor by a retention device (see e.g. document D13, Figures 1 to 3).

4.2 Since the terms "*at least some portion*" and "*at least one waste product*" in step (b) neither quantify the amount/percentage of retained cells nor specify the

waste product, step (b) encompasses any amount of cells and any type of waste product generated by CHO cells.

5. The second growth phase is further specified in step (c) in that the CHO cells in "*the cell culture*" are grown "*to a second critical level*" which is reached at:

- "*a cell density of 5 million to 40 million cells per milliliter, or,*"
- "*at day 2 to day 7 of the cell culture*".

Thus two further alternative parameter ranges (cell density or cell culture duration) specify that the CHO cell culture has reached its "*second critical level*".

6. Step (d) specifies that "*a polypeptide production phase*" is initiated by "*a change in temperature, pH or osmolality of the cell culture or combination thereof*".

6.1 The actual time point of initiating the production phase is not defined in step (d) of claim 1, except that it must be after the CHO cells have reached their second critical level (see step (c)). Thus, the production phase may be initiated either immediately after the cell culture has reached the second critical level, or at some undefined time point later.

6.2 Furthermore the term "*change*" in step (d) is not quantitatively defined but qualitatively only in that this change must result in "*initiating*" the production phase. This requires a change of temperature, pH or osmolality either alone or in any combination that is suitable for this purpose.

6.3 The meaning of "*a polypeptide production phase*" in step (d) of claim 1 is a matter of dispute.

6.4 The opposition division (see decision under appeal, point 4.1) and the respondent held that this term related to the phase of the cell culture where the majority of protein is produced.

6.5 The board does not agree. It is undisputed that CHO cells in the claimed method produce some protein already during the cell growth phases (steps (a) to (c), see e.g. document D72, point 3.1, first sentence).

6.6 It is moreover common general knowledge that "*the protein synthesis rate in the bioreactor is directly proportional to the number of viable and productive cells (7), the higher cell density in perfusion bioreactors results directly in a higher protein production rate*" (see e.g. document D13, page 388, first paragraph).

6.7 Based on this general understanding, the board agrees with the respondent's expert Dr. Hiller that "*in order to maximize overall productivity from a cell culture, it may be desirable to initially grow the cells in the culture under conditions which optimize the rate of cell growth and division (to rapidly create a large number of cells in the culture), and then to change the cell culture conditions so the rate of cell division in the culture is reduced, and the rate of production of proteins from the cells in the culture is increased. This later stage of the just-described cell culture process (i.e. the stage when the rate of division of the cells is decreased and the rate of production of protein by the cells is increased) is referred to as a "polypeptide production phase"....Rather, the "polypeptide production phase" is the stage when conditions in the culture are set to favor protein*

*production from the cells, as opposed to cell division*" (see document D72, point 3.1, emphasis added).

6.8 Thus the skilled person construing "*a polypeptide production phase*" in step (d) of claim 1 in its broadest technically sensible manner relates this term to the culture phase wherein after a trigger the production rate of the antibody is increased compared to the cell growth phases. In the absence of any further indications in claim 1, any increase of the production rate suffices after changing at least one of the conditions indicated in step (d), even a minor one compared to the growth phase irrespective of the amount of antibody produced. Since, moreover, the production rate directly affects the antibody amount generated (the yield) and claim 1 does not specify the end point of the production phase either (i.e. its overall duration), claim 1 encompasses as embodiments methods that produce high and low antibody yields. Further, since the duration of the production phase is not defined in claim 1, claim 1 lacks also a requirement that the majority of the antibody has to be produced during the production phase.

7. Reading steps (c) and (d) of claim 1 in conjunction, these steps define the requirements for initiating the protein production phase (i.e. reaching a "*second critical level*" followed by a change of temperature, pH or osmolality either alone or in any combination) while the actual start and end of the production phase is left open, i.e. the production duration.

8. Nor is the end point of perfusion culturing defined in claim 1. This interpretation is in line with dependent claim 11 which reads: "*wherein the step of maintaining*

*cells in fed-batch culture is initiated after a period of time has elapsed since the cell culture reached the second critical level*" (emphasis added). The respondent submitted that the skilled person would rule out unreasonable long durations of perfusion culturing in the claimed method. Since it is unclear which time span is thereby covered, the board considers that any duration of perfusion culturing normally applied in the art falls within the scope of claim 1.

9. Step (e) specifies that cells are maintained "*in a fed-batch culture during at least some portion of the polypeptide production phase*". The term "*at least some portion*" in step (e) indicates that any part, even a minor one of the production phase must lie within fed-batch culturing. This implies that the production phase specified in steps (c) and (d) may start and well extend into perfusion culturing for an undefined period of time prior to the start of fed-batch culturing. Neither the start nor the end points of fed-batch culturing are specified in claim 1. Consequently, the overall duration of fed-batch culturing is left open, like that of perfusion culturing (point 8 above).

10. In summary, the first growth phase as specified in claim 1 lasts until the CHO culture has reached a first critical level which is followed by a second perfusion-based growth phase. Lastly, an antibody production phase of undefined length is initiated by a trigger after the cell culture has reached a second critical level which necessarily involves at least in part fed-batch culturing.

*Inventive step - claim 1*

*Suitability of document D1 in the assessment of inventive step*

11. The respondent argued that document D1 was not a suitable prior art for assessing inventive step of the method of claim 1 because it was directed to a different purpose (production of a cytotoxic protein instead of an antibody). Moreover, the selection of document D1 was based on hindsight.

12. The board does not agree.

12.1 Although the cell culture method of claim 1 is directed to the production of an antibody, this method is not specific to, or particularly suitable for, producing antibodies. Paragraph [0058] of the patent states in this context that "*The present invention may be used to culture cells for the advantageous production of any therapeutic protein, such as pharmaceutically or commercially relevant enzymes, receptors, receptor fusions, antibodies (e.g., monoclonal and/or polyclonal antibodies), antigen-binding fragments of an antibody, Fc fusion proteins, cytokines, hormones, regulatory factors, growth factors, coagulation / clotting factors, or antigen-binding agents. The above list of proteins is merely exemplary in nature, and is not intended to be a limiting recitation*" (emphasis added). Since the list of proteins cited in the patent is exemplary, the claimed method does not exclude the production of a cytotoxic protein as disclosed in document D1.

12.2 There are also no reasons apparent to the board that the method disclosed in document D1 is technically not suitable for the production of an antibody. Nor have any reasons in this regard been forwarded by the respondent. The method of document D1 and the claimed

method are thus directed to the same general purpose, i.e. protein production.

12.3 Moreover, document D1 discloses the use of CHO cells in a single cell culturing process (STR#1.1 to STR#1.4) for the production of a cytotoxic protein. After the culture has reached a first critical level ("*1.1 x $10^6$ cells/ml*", i.e. 1.1 million cells per millilitre), perfusion culturing starts that after reaching a second critical level ("*20 x $10^6$ cells/ml*", i.e. 20 million cells per millilitre) is followed by a fed-batch culturing (see Table 1 on page 298 and Figure 2). Thus the so called "STR#1.1 / STR#1.4" cell culturing process of document D1 and the claimed method share many relevant technical features too. Consequently, the STR#1.1 / STR#1.4 process of document D1 represents a suitable springboard for assessing inventive step.

13. Irrespective of these considerations, it is established case law that a claimed invention must be non-obvious having regard to any prior art and, if an inventive step is to be denied, the choice of starting point needs no specific justification (Case Law of the Boards of Appeal of the EPO, $10^{th}$ edition 2022, ("Case Law"), I.D.3.1, in particular, T 261/19, Reasons 2.5).

14. It is uncontested that the claimed method differs from the so called STR#1.1 / STR#1.4 process of document D1 in (1) the production of an antibody and (2) in step (d), i.e. the initiation of a production phase by, for example, a temperature shift.

15. The issue is contentious however whether or not these two distinguishing features result in technical effects that render the claimed method advantageous over the whole scope claimed. The respondent argued that in

particular the initiation of the production phase as defined in step (d) of claim 1 had the effect that the overall time required for producing the antibody was shorter and resulted in higher yields compared to document D1. In other words, due to the production initiation step the claimed method produced more protein in less time.

16. The board does not agree.

17. It is established case law that it must at least be credible based either on the data disclosed in the patent or on common general knowledge that the alleged advantageous effects that have been ascribed to a distinguishing feature have been achieved across the whole scope claimed (Case Law, I.D.4.3.1).

*Step (d) of claim 1 as distinguishing feature*

18. Since comparative data between the claimed method and that of document D1 are not on file, the issue to be assessed is whether it is at least credible based either on the data of the patent and/or the skilled person's common general knowledge that the initiation of the protein production as defined in step (d) of claim 1 produces more protein in less time over the whole scope claimed when compared to the method of document D1.

18.1 The patent discloses in Examples 2.2 to 2.4 that the final yield of antibodies obtained by growing CHO cells under perfusion followed by fed-batch was high (see Figures 8, 12 and 18). The cells were grown for up to 18 days under specific experimental conditions. Examples 2.2 to 2.4 of the patent use a temperature shift for triggering the production phase (temperature

drop from "*37°C to 31°C*", see e.g. patent, column 23, lines 9 and 44), while none of these working examples disclose the use of a control, i.e. a culturing without a temperature shift. In the absence of such a control, however, a potential contribution of the temperature shift on cell culture duration and antibody yield cannot be derived from the patent.

18.2 Are the asserted effects credible in light of the skilled person's common general knowledge?

18.3 As regards the yield, a potential effect of the temperature shift reported in Examples 2.2 to 2.4 of the patent on the antibody yield might be credible in light of the skilled person's common general knowledge (see the abstracts of documents D6 and D18).

18.3.1 However, as set out above under claim construction (point 6.8), the claimed method is not directed to a particular antibody yield because claim 1 neither specifies the duration of the production phase nor the increase in the antibody's production rate after initiating the production phase. Claim 1 comprises as an embodiment a method that produces low antibody yields only.

18.3.2 In view of these considerations, the yield shown in Figures 8, 12 and 18 of the patent cannot be generalised for all embodiments falling within the scope of claim 1 and therefore can not be taken into account in defining the objective technical problem. In view thereof the question of whether or not document D1 discloses that during fed batch culturing the yield of the cytotoxic protein goes down or not (declaration D89) can be left unanswered since this is irrelevant for the present case where yield is not decisive.

18.4 As regards the duration of cell cultivation after initiating the production phase as defined in step (d) of claim 1, the following is relevant.

18.4.1 As set out above (point 18.1), an effect of the protein production initiating step (d) of claim 1 on the overall cell culture duration cannot be derived from the experimental data provided in the patent, nor by any other data of the available prior art.

18.4.2 The respondent argued that the culture duration shown in Figure 2 of document D1 lasted for about 70 days which was significantly longer than the maximum of 18 days disclosed in Figures 8, 12 and 18 of the patent. However, since the cell culturing in document D1 and in the patent are performed under different experimental conditions that are not comparable *per se* and moreover depend on the subjective aim the skilled person intends to achieve, the mere disclosure of a shorter or longer cultivation alone cannot support a technical effect.

18.4.3 Irrespective thereof, as set out above under claim construction (point 7), the duration of the production phase is not specified in claim 1, i.e. the claimed method encompasses short and long production phases. Accordingly, methods that cultivate CHO cells for several weeks are encompassed by claim 1. Contrary to the respondent's view, this is not unreasonable since perfusion cultures lasting for weeks are known from the prior art (see e.g. document D1, Figure 2). The short culture durations shown in Figures 8, 12 and 18 of the patent cannot therefore be generalised for all embodiments falling within claim 1. Also for this reason, the respondent's arguments are not persuasive.

*Antibodies as distinguishing feature*

19. As regards the production of an antibody instead of a cytotoxic protein, the respondent has not forwarded any unexpected technical effects that could be ascribed to this distinguishing feature. This difference thus amounts to the production of a mere alternative protein.

*Objective technical problem*

20. Consequently, an advantageous technical effect cannot be ascribed to all embodiments falling within the scope of claim 1. It is established case law that in these circumstances the technical problem to be solved must be formulated in less ambitious terms (Case Law, I.D. 4.4.1).

21. Accordingly, the objective technical problem to be solved by the method of claim 1 resides in the provision of an alternative cell culture method for recombinant protein production.

22. In view of the experimental data disclosed in the patent, the board is satisfied that this problem is solved by the claimed cell culture method.

*Obviousness*

23. It remains to be assessed whether the skilled person starting from the method of document D1 and faced with the technical problem defined above would have arrived at the method of claim 1 in an obvious manner.

24. Since the problem to be solved resides in the provision of an alternative method, the skilled person would

consult other commonly known cell culture-based methods used for recombinant protein production.

25. It is undisputed that the use of a temperature shift in a CHO cell culture producing a recombinant protein belongs to the skilled person's common general knowledge (see e.g. documents D6 and D18, abstracts). The same applies to the use of CHO cells for the production of antibodies (see, e.g. document D12, page 375, last paragraph). Furthermore the production of an antibody instead of a cytotoxic protein represents a mere alternative (point 19 above). It is established case law that the simple act of arbitrarily selecting one among equally obvious alternative compounds (here antibody instead of cytotoxic protein) is devoid of any inventive character (Case Law, I.D.9.21.9, in particular T 892/08, Reasons 1.7). In light thereof the skilled person starting from document D1 and taking common general knowledge into account would have arrived at the method of claim 1 in an obvious manner.

26. The respondent argued that document D1 lacked a pointer for using a temperature shift in general and in particular for such a shift that initiated the production phase only after the culture has reached its second critical level (step (c) of claim 1). Furthermore, document D1's teaching rather led the skilled person away from combining perfusion with fed-batch culturing for protein production in CHO cells due to an observed decreased cell viability and increased cell lysis during fed-batch culturing.

27. The board does not agree.

28. It is established case law that for solving a technical problem as defined above, a pointer in the closest

prior art (here document D1) is not needed unless the closest prior art teaches away from the solution (Case Law, I.D.4.5, in particular T 1179/16, Reasons 3.4.4).

29. As regards the asserted teaching away in document D1 of using a combined perfusion / fed-batch culturing, document D1 discloses repeatedly that perfusion culturing may be combined with fed-batch for optimising the yield (abstract, penultimate sentence, page 298, last paragraph and page 299, last sentence).

29.1 In the paragraph bridging pages 298 and 299, document D1 states that after switching from perfusion to fed-batch "*the loss of culture viability was significant*" and that therefore enzymes could be released that "*can negatively affect the product quality at a prolonged residence time in the reactor*".

29.2 However, this statement represents no absolute bar for the skilled person in not combining perfusion culturing with fed-batch. It merely suggests that "*Further analysis of the (glyco-)protein quality would be important, since investigations on product integrity were not performed in this study*" (see paragraph bridging pages 298 and 299). This teaching rather raises the skilled person's awareness that if quality is essential, additional studies ensuring the product's quality should be performed. Document D1 therefore does not teach away from using a combined perfusion / fed-batch culturing but at most invites the skilled person in assessing the product's quality if needed.

30. As regards the specific time point for initiating protein production as defined in step (d) of claim 1, i.e. only after the culture has reached its second critical level, the following is relevant.

30.1 The patent neither mentions nor provides experimental evidence that this specific time point for initiating the protein production is associated with a technical effect. Examples 2.2 and 2.3 of the patent rather disclose that it makes no difference whether the protein production is initiated during perfusion or at the end of perfusion. Nor does claim 1 specify the actual time point of initiating the protein production phase after reaching the second critical level (point 6.1 above). The production phase can thus be triggered at any time after the culture has reached this level, i.e. immediately or later, which implies that both events (second critical level and initiation) are independent of each other and therefore technically not linked.

30.2 The time point *per se* of initiating protein production as defined in claim 1 cannot therefore establish the presence of an inventive step either.

31. The respondent submitted further as a secondary indication of inventive step that several years lay between the publication of document D1 and the filing of the patent in suit during which no other document reported on the combined use of perfusion and fed-batch culturing in conjunction with a temperature shift. However, in the absence of other reasons, the sole non-disclosure of a combined perfusion / fed-batch cell culturing using a temperature shift in the relevant period of time does not qualify as a secondary indication that the skilled person would not use a temperature shift in an alternative cell culture method for protein production when starting from the method of document D1 (Case Law, I.D.10.1).

32. The method of claim 1 is thus not inventive over the teaching of document D1 in combination with common general knowledge. The main request does not comply with the requirements of Article 56 EPC.

*Auxiliary request 1*

33. The method of claim 1 differs from that of the main request in that the second critical level in step (c) has been limited to a duration from "*at day 2 to day 7 of the cell culture*".

*Inventive step - claim 1*

34. The limitation of step (c) in claim 1 to a specific duration as the sole parameter indicating that the culture has reached its second critical level merely requires that cells have been cultivated for two to seven days before the protein production phase is initiated. This specific duration is not associated with any parameter that is relevant for protein production, for example, cell density (point 6.6 above). Rather the period of days indicated in step (c) of claim 1 is directed to a mere passing of time which in itself has no technical effect. Since this feature is hence arbitrary because it does not contribute to the solution of the technical problem (the provision of an alternative method for protein production), it is disregarded in assessing inventive step (Case Law, I.D. 9.6).

35. Accordingly the reasoning provided above for claim 1 of the main request under lack of inventive step applies to the method of claim 1 of auxiliary request 1 too.

36. The respondent submitted that the time period indicated in step (c) of claim 1 had the advantageous effect that in cell cultures of known behaviour, particular parameters, such as cell density, no longer had to be controlled before the protein production was initiated. This saved time and costs.

37. This is not persuasive. The method of claim 1 is not limited to cell cultures with "known" behaviours, nor do cell cultures behave "normally" under all circumstances. Further, due to the "comprising" language, claim 1 does not exclude the determination of additional cell culture-related parameters. Nor does this limitation affect the overall duration of perfusion culturing. Amended step (c) of claim 1 specifies the duration of perfusion culturing only in so far as it indicates that the CHO cell culture has reached its second critical level (point 5 above). However since the time point of initiating the production phase is not specified in step (d) of claim 1, the end point of perfusion is left open for the reasons indicated above (points 6.1 and 7 to 9) and hence the overall duration of perfusion culturing.

38. Auxiliary request 1 does not comply with Article 56 EPC.

*Auxiliary request 2*

39. Claim 1 of auxiliary request 2 differs from that of auxiliary request 1 in that the initiation of the protein production phase in step (d) is further limited in that it must comprise a temperature shift.

*Inventive step - claim 1*

40. Since the reasoning under lack of inventive step provided above for the method of claim 1 of the main request already includes the use of a temperature shift, this reasoning applies to the method of claim 1 of auxiliary request 2 too.

41. Auxiliary request 2 does not comply with Article 56 EPC either.

*Auxiliary request 3*

42. Claim 1 of auxiliary request 3 differs from that of auxiliary request 2 in that the claimed method is further limited in that at least one waste product "*is removed by passing the spent medium through an ultrafiltration device*".

*Inventive step - claim 1*

43. The respondent submitted that the patent demonstrated in Examples 2.3 and 2.4 that the use of an ultrafiltration ("UF") device allowed the production of higher antibody titres in shorter time and the production of higher overall yields compared to a microfiltration ("MF") device (see Figures 12 and 18). Since MF devices were comparable to the ultrasound cell retention device of document D1 (both were unable to retain antibodies but only retained larger entities, such as cells), the claimed cell culture method was improved over that of document D1.

44. The board does not agree.

45. Document D1 discloses an ultrasound-based cell retention device (page 294, last paragraph) but is silent on MF and UF cell retention devices. Comparative

data concerning the retention properties of these three devices are not on file, so that it is unknown whether an ultrasound-based device has the properties of a MF device as asserted by the respondent. Irrespective of the fact that therefore a comparison between an ultrasound-based cell retention device and an UF device and their effects on culture time, titre and yield can not be made either, the following is relevant.

46. As regards Example 2.3 of the patent, the data disclosed in Figure 12 are obtained by culturing cells under specific experimental conditions. Example 2.3, for example, discloses in paragraph [0071] the use of a "*bioreactor operating at high perfusion rate, except the recirculation loop contained an ultrafiltration device (UF) hollow fiber with a cut-off of 50,000 daltons. This device retained nearly 100% of the polypeptide product (i.e., the anti-IL-22 antibody)*" (emphasis added).

46.1 The UF device indicated in claim 1 is not limited by a cut-off size of 50,000 daltons ("Da"). The patent discloses in this context that UF filters may have pore sizes up to a cut-off size of "*750,000 daltons*" (paragraph [0036]) and that antibodies have a size of "*typically about 150,000 daltons*" (paragraph [0039]). This explains why the antibodies in Example 2.3 are unable to pass the UF device with a cut-off size of 50,000 Da (the pores are simply too small), while antibodies would easily pass an UF device with a cut-off size of 750,000 Da due to the larger pore size. Already for this reason the results of Example 2.3 cannot be generalised to all UF devices falling within the scope of claim 1.

46.2 Irrespective thereof no data are available, neither from the patent nor from the prior art, that show that the use of UF filters in general allows the production of higher antibody titres in shorter cultivation times. As regards the yield, Example 2.3 discloses that the antibody titres at the end of the cultivation are the same for MF and UF devices, despite the larger pore size of MF filters (pore sizes range from "*0.1 to 10 µM*", patent, paragraph [0036]). Consequently, the pore size is irrelevant for the actual yield obtained. Irrespective thereof, for the reasons indicated above (point 6.8), claim 1 is not limited to a particular antibody yield. This is not changed by the use of an UF device as indicated in claim 1.

46.3 Nor is claim 1 limited to a high perfusion rate. Since it cannot be excluded that growth conditions affect the antibody titre, its yield and the culture duration, the results of Example 2.3 cannot be generalised to all embodiments falling within claim 1.

47. As regards Example 2.4, the patent discloses that the conditions used for producing the antibody differ not only with respect to the UF and MF device used but also in that *"One bioreactor used normal medium and had the recirculation loop attached to a MF device (R1), while another bioreactor used a more concentrated medium formulation and had the recirculation loop attached to a UF device (R2)*" (paragraph [0072]). Thus the conditions compared in Example 2.4 differ also with respect to the medium in which the CHO cells are grown: in the experiments with an UF device, the cells are grown in "*a more concentrated*" medium, while in the MF experiment, cells are grown in "*normal*" medium only. Since it cannot be excluded that the better growth conditions for CHO cells in the UF device experiment

affected the higher antibody yields disclosed in Figure 18 of the patent, no conclusions can be drawn about any potential effect on yield in using an UF device versus a MF device. Thus Example 2.4 cannot support an advantageous effect in using UF versus MF devices either.

48. In the absence of data supporting an advantageous effect in using UF devices compared to MF devices in a cell culture-based production of an antibody, it is also not credible that UF devices might have any advantageous effects compared to the ultrasound retention device of document D1. All these devices retain cells in the perfusion reactor to obtain higher cell densities. Moreover, as set out above (point 6.6), a higher number of viable cells in the reactor is positively correlated with the amount of recombinant protein produced. In these circumstances the technical problem to be solved remains the same as that defined above (point 21) for the method of claim 1 of the main request.

*Obviousness*

49. Therefore, since the problem to be solved resides in the provision of an alternative method, the skilled person would look at other commonly known cell culture-based methods used for protein production (point 24 above). Document D13 is a textbook which represents the common general knowledge of the skilled person at the relevant filing date of the patent. As argued by the appellants, this document, for example, discloses the use of UF-based hollow fibre retention devices in perfusion reactors in the production of antibodies (page 390, fourth paragraph). The skilled person starting from document D1 and taking common general

knowledge into account would thus have arrived at the method of claim 1 in an obvious manner.

50. Auxiliary request 3 does therefore not comply with Article 56 EPC either.

51. Since none of the requests on file are based on an inventive step over the teaching of document D1, the patent must be revoked.

**Order**

**For these reasons it is decided that:**

The decision under appeal is set aside.

The patent is revoked.

The Registrar: The Chair:

C. Rodríguez Rodríguez T. Sommerfeld

Decision electronically authenticated