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Datasheet for the decision of 29 July 2022

Case Number: T 0875/19 - 3.3.08

Application Number: 10183677.3

Publication Number: 2343362

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Language of the proceedings: EN

Title of invention:
Improved process for the culturing of cells

Patent Proprietor:
Patheon Holdings I B.V.

Opponents:
Baxalta GmbH
Boehringer Ingelheim Pharma GmbH & Co. KG/
Boehringer Ingelheim International GmbH
Repligen Corporation

Headword:
Cell culturing/PATHEON

Relevant legal provisions:
EPC Art. 54, 56, 83, 87, 123(2)
RPBA Art. 12(4)

Keyword:
Late filed facts - admitted - (no)
Main request - admitted - (yes)
Main request - requirements of the EPC met - (yes)

Decisions cited:
G 0001/03, G 0001/15

Catchword:

Europäisches Patentamt
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Beschwerdekammern

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Case Number: T 0875/19 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 29 July 2022

Appellant: (Opponent 1)	Baxalta GmbH Thurgauerstrasse 130 8152 Glattpark (Opfikon) (CH)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted on 22 January 2019 concerning maintenance of the European Patent No. 2343362 in amended form.**

Composition of the Board:

Chairman B. Stolz
Members: M. Montrone
D. Rogers

Summary of Facts and Submissions

I. The appeal is against the decision of an opposition division to maintain the European patent No. 2 343 362 in amended form. This patent is based on European patent application No. 10 183 677.3 which is a divisional of the earlier patent application No. 07765049.7 published as International patent application WO 2008/006494 ("earlier patent application").

II. With their statement of grounds of appeal, opponent 01 ("appellant") submitted objections against the subject-matter of the main request under added subject-matter, insufficiency of disclosure, lack of priority entitlement, lack of novelty and inventive step. In support of their case *inter alia* new documents D61 and D62 were submitted.

III. In reply, the patent proprietor ("respondent") submitted the main request as maintained by the opposition division, and auxiliary requests 1 to 6. These auxiliary requests correspond to auxiliary requests IV, V, VII, VIII, IX and XI, respectively, as submitted during the first instance proceedings.

IV. In reply, the appellant submitted further arguments and documents.

V. In reply to the summons to oral proceedings, opponent 02 informed the board that they would not attend the oral proceedings. Opponent 03 had already withdrawn their opposition during the first instance proceedings.

VI. The appellant requested a re-scheduling of the present case, and of the two related cases T 804/19 and T 513/20 to dates that were closer together. This request was not granted due to the board's workload.

VII. In a communication in preparation of oral proceedings, the parties were informed of the board's provisional, non-binding opinion.

VIII. In reply, the appellant informed the board that they would not attend the oral proceedings.

IX. Oral proceedings before the board were held on 29 July 2022, in the absence of the appellant and of opponent 02 as announced. During the oral proceedings, the respondent withdrew the main request, and auxiliary request 1 became their new main request.

X. Claim 1 of the main request (filed as auxiliary request 1 under cover of a letter dated 16 October 2019) reads:

"1. Process for the culturing of eukaryotic cells in a reactor in suspension in a serum free cell culture medium, wherein the cells produce a recombinant protein,
wherein at least one cell culture medium component is fed to the cell culture and wherein the cell culture comprising the cells, the recombinant protein and cell culture medium is circulated over a hollow fiber filter in a flow substantially parallel to a surface of said filter resulting in a liquid outflow and a flow which contents are kept in or fed back into the reactor, wherein the filter has a pore size suitable to separate the recombinant protein from substances having a lower molecular weight than the recombinant protein and wherein the liquid outflow consists essentially of

components having a molecular weight lower than that of the recombinant protein and
wherein the recombinant protein is retained in or fed back into the reactor".

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

D2: DE 10120835 (published 7 November 2002);

D5: Flickinger, M.C. *et al.*, Bioprocess Engineering, 1990, Vol. 5, 155-164;

D7: Furey J., Genetic Engineering News, 2002, Vol. 22(7), 62-63;

D9: Koros W. J. *et al.*, Pure & Applied Chemistry, 1996, 1479-1489;

D10: Millipore Technical Brief "Protein Concentration and Diafiltration by Tangential Flow Filtration", 2003;

D13: Takazawa, Y. *et al.*, Cytotechnology, 1988, Vol. 1, 171-178;

D15: US 4,806,484 (published 21 February 1989);

D16: US 5,286,646 (published 15 February 1994);

D17: US 6,544,424 (published 8 April 2003);

D20: WO2005/095578 (published 13 October 2005);

D21: Matsumura M. and Nayve F.R., Cytotechnology, 1995, Vol. 18, 35-50;

D22: Refine Technology, Co., "ATF system", Operating Manual for Models ATF-4 and ATF-6, 2000;

D25: WO2008/152075 (published 18 December 2008);

D27: EP2041259 (parent of the patent in suit);

D28: EP2634242 (divisional of the patent in suit);

D29: EP2634243 (divisional of the patent in suit);

D30: EP2634250 (divisional of the patent in suit);

D31: Presentation "The human PER.C6® manufacturing platform", published 10 May 2007;

D44: Declaration John Bonham-Carter, dated 19 December 2017;

D61: "Selection guide: Separation products for centrifuged and tangential flow filtration", Pall Corporation;

D62: Avonex (Interferon beta-1a) prescription information.

XII. The appellant's written submissions, insofar as relevant to the present decision, may be summarised as follows:

Admission into the appeal proceedings of the main request (submitted as auxiliary request 1), of documents D22, D61 and D62, and a new argument under insufficiency of disclosure based on document D7

The auxiliary requests filed in reply to the statement of grounds of appeal were not convergent, and should thus not be admitted into the proceedings.

Document D22 was not admitted by the opposition division into the proceedings. The opposition division erroneously applied the "up-to-the-hilt" standard, and not the appropriate balance of probabilities standard for deciding on the public availability of document D22. The document was a power point presentation on alternating tangential flow (ATF) technology given every month in the years 2000-2004 during sales presentations to companies in the UK and Ireland. The audience was under no confidentiality constraints as confirmed by the declaration of Mr. Bonham-Carter (see D44). While document D22 was not a commercial brochure, it served the same purpose, i.e. ATF's promotion.

Documents D61 and D62 were already filed in parallel proceedings concerning the applications EP2041259 and EP2634242, respectively. Document D61 was newly cited in the context of inventive step in support of selecting a membrane with appropriate retention characteristics (see page 48, fourth to sixth paragraph of the statement of grounds of appeal). Document D62 was newly cited in support of an argument under insufficiency against auxiliary request 2 in the appeal proceedings (see page 76, last paragraph of the statement of grounds of appeal: auxiliary request 5 cited here corresponds to auxiliary 2 in the appeal proceedings). These documents were relevant for auxiliary requests limited by a filter with a molecular weight cut off size.

Main request

Added subject-matter - claim 1

Claim 1 comprised added subject-matter because the application as filed provided no basis for the combination of features "*eukaryotic cells*", "*hollow fiber filter*", "*substantially parallel*", "*recombinant protein*", and "*serum-free*".
While page 3, lines 7 to 13 of the application as filed disclosed "*eukaryotic cells*", a selection from a list of alternatives had to be made.
Page 5 and claim 5 of the application as filed mentioned a "*hollow fiber filter*". However, this filter was selected from a list of alternatives.
The term "*substantially parallel*" was mentioned on page 6, lines 24 to 27 of the application as filed, however, in a list of two alternatives.
The feature "*recombinant protein*" was selected from a further list disclosed on page 10, last paragraph to page 11, first paragraph of the application as filed.
The feature "*serum-free*" was selected from another list of two alternatives.
None of these features were disclosed in individualised form in the application as filed. The selection from multiple lists generated a new combination of features.

Furthermore, the application as filed provided no basis for the term "*consists essentially of*" in claim 1, which solely disclosed the expression "*the liquid outflow will essentially only contain*" (see page 5, line 33 and 34). Both statements were not equivalent. While the amended claim defined what the flow was made up of due to the use of "*consists of*", the original disclosure indicated merely the presence of a flow and defined its content.

Likewise the cells cited in claims 3 to 6, and the antibody of claim 7 represented selections from a list.

Sufficiency of disclosure

The breadth of the claim represented an undue burden for the skilled person. The claimed process referred to any kind of culturing, tangential flow and cells. The patent application, however, disclosed one specific process only.

Furthermore, the claimed process comprised non-working embodiments. Documents D7 and D13 disclosed a process that fell within the scope of claim 1. Document D7 indicated filter clogging problems despite the use of tangential flow filtration (TFF). Likewise document D13 reported that in initial experiments cells caused filter clogging despite the use of TFF (see page 176, column 1, second paragraph).

Priority

None of the priority documents provided a basis for the subject-matter of the main request for the same reasons set out above under added subject-matter. The main request was thus not entitled to priority, and the relevant date was the filing date of the patent application, i.e. 4 July 2007.

Novelty

Document D2 described the production of monoclonal antibodies from mammalian cells, including IgG. The cells were cultured in suspension. Fresh medium was added while waste products were removed via a hollow fiber filter located in the culture medium of the

reactor. The filter's MWCO was preferably in the range of 3 kDa to 10 kDa (see paragraphs [0001], [0010], [0015], [0028] and [0035]). The fresh medium circulated inside the filter. Alternatively an alternating flow direction of the fresh/waste medium inside the filter was applied mediated by a reversed pressure gradient across the filter membrane (see column 3, lines 25, 26, paragraph [0010], point (c), and [0031]). The cell culture in the reactor was continuously moved by an impeller (see paragraph [0023]). Since the filter was immersed in this rotating culture medium it was "*inevitable that there is a tangential flow when circulating the medium over the filter*", as likewise implied by the German verb "*umfließt*". Document D2 disclosed thus all features of the process of claim 1.

Figure 1 of document D13 disclosed the claimed process. The cell culturing was performed in serum-free medium.

Document D15 disclosed an airlift bioreactor for culturing suspended hybridoma cells producing a monoclonal antibody (see column 1, lines 19 to 29, column 2, second and third paragraph). The reactor set-up used an external hollow fiber filter (designated as "*external cross flow filter*") for exchanging fresh nutrients and waste products in the cell culture (see column 1, third paragraph, and Figure 1). Fresh medium and the culture medium were pumped through the filter in a tangential flow (see Figure 1). Due to the hollow fiber's different cross-sectional area of the lumen and shell space it was inevitable that the pumps created a pressure gradient across the membrane which caused the nutrient/waste exchange. This pressure gradient resulted in a tangential flow filtration, and not in a dialysis-based exchange.

Document D16 used a very similar cell culturing system to that of document D15 (see Figure 2). Accordingly, the same reasoning applied.

Documents D27 to D30 were the parent application or divisional applications of the patent in suit. Since the priority of the patent was not valid these applications represented "*poisonous divisionals*" which were detrimental to the process of claim 1.

Inventive step

Claim 1 of auxiliary request 1 suffered from the same deficiencies under inventive step as the main request. Each of documents D2, D5, D13, D15, D16, and D21 represented the closest prior art. Furthermore, hollow fibers and their benefits were known, for example, from documents D7 and D17.

Document D2 disclosed a perfusion culturing of cells for the production of antibodies. The claimed method differed therefrom in using a tangential flow for filtration. The use of TFF, however, was not inventive since TFF was widely applied in cell culturing.

Document D5 disclosed a perfusion culturing of cells for the production of an antibody (see abstract). The process used a membrane filter operated by tangential flow for retaining cells and antibodies in the cell culture (see Figures 2 and 15). The filter had a pore size of 0.20 micron or 0.65 micron. The effective pore size of the filter was smaller because serum proteins in the medium caused a gel polarisation on the filter surface. This resulted in a filter with retention properties similar to those of the 10 kDa membrane

disclosed in document D13 (see page 163, column 1, second paragraph).
The claimed method differed from document D5 in using serum-free medium for the cultivation of cells. Since there was no beneficial effect associated with this difference, the problem to be solved remained the provision of an alternative process. The culturing of cells in serum-free medium was well known in the art, including the general motivation for avoiding the use of serum in cell culture media.
The skilled person performing the process of document D5 in serum-free medium was aware that the filter had to be changed under these circumstances since the antibody's retention was caused by gel polarisation, i.e. the presence of serum proteins. Suitable filters were, for example, mentioned in documents D10 and D13.

Document D13 disclosed a perfusion culturing of hybridoma cells in serum-free medium for producing an antibody. The cell culture passed through a membrane filter (Pellicon cassette) with a MWCO of 10 kDa, and the concentrated product was fed back into the reactor (see abstract, Figure 1, page 173, column 1, second paragraph). In initial experiments solely a cell settling zone within the reactor was used to reduce the amount of cells circulating over the filter. The flow out cells, however, caused filter clogging (see page 176, column 1, second paragraph). A second experimental set-up used an additional cell trap to prevent filter clogging. This trap did not remove all cells from the cell culture. Even if it did, it was obvious to replace the cell trap with an ATF system. ATF was an established system, commercially available, and known to prevent filter clogging (see e.g. document D17, column 1, lines 49 to 57, column 3, lines 42 to 67). Thus, the claimed method was an obvious alternative to

the second experimental set-up reported in document D13.

Document D21 disclosed a process for culturing mouse hybridoma cells producing an antibody in serum-free medium (see page 36, column 1, second but last, and last paragraph). The cells and the IgG were retained in the reactor through a so called dead-end filtration mediated by a membrane filter with a MWCO of 10 kDa that was fixed at the bottom of the reactor. The difference between the claimed method and document D21 was the use of a substantially parallel flow over the filter surface instead of using dead-end filtration. Since no effect was ascribable to this difference, the technical problem to be solved remained the provision of an alternative method. The use of TFF instead of dead-end filtration as an alternative was obvious for the skilled person.

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

Admission into the appeal proceedings of the main request (submitted as auxiliary request 1), of documents D22, D61 and D62, and a new argument under insufficiency of disclosure based on document D7

The main request was submitted in reply to the appellant's statement of grounds of appeal as auxiliary request 1. Moreover, this claim request was already submitted during the first instance proceedings. It should thus be admitted into the appeal proceedings.

The opposition division's decision was correct in not admitting document D22 into the proceedings. This document was a product presentation by Refine

Technology, i.e. opponent 03 in these proceedings. This document was not publicly available under Article 54(2) EPC because it stated on its front page "*Copyright 2000 - Confidential Information Enclosed*". The declaration D44 did not provide evidence to the contrary since Mr. Bonham-Carter was an employee of opponent 03. Document D22 was not a widely distributed commercial brochure since the presentation was given to potential customers under confidentiality constraints.

Documents D61 and D62 were newly cited in the appeal proceedings, although the main request was already filed at the first instance proceedings. These documents should thus not be admitted.

The appellant submitted under insufficiency that the continuous pore size reduction of the filter by gel polarisation as disclosed in document D7 led to non-working embodiments. This argument was raised for the first time in the appeal proceedings, despite the main request was already filed during the first instance proceedings. This argument should thus not be admitted.

Main request

Added subject-matter - claim 1

The claims of the main request comprised no added subject-matter.
The process of claim 1 had primarily a basis in claim 1 as filed, and in the paragraph that bridged pages 1 and 2 of the application as filed. In particular, the application as filed provided a basis for the following features: the term "*eukaryotic cells*" was disclosed on page 3, lines 7 to 13; the preferred use of a "*hollow fiber filter*" was disclosed on page 5, line 22, and in

claim 5; the term "*substantially parallel*" was mentioned on page 6, lines 24 to 26; the "*recombinant protein*" was disclosed on page 10, line 31, and in claim 4; "*serum-free*" was disclosed on page 13, lines 4 to 7; the replacement of the expression "*essentially only contains*" by "*consists essentially of*" had a basis on page 5, line 33 to page 6, line 1, since both expressions had an identical meaning.
The cells cited in claims 3 to 6 were disclosed in claim 3, and on page 3, lines 15 to 20.
Lastly, the term "*antibody*" in claim 7 was disclosed in claim 4.

Sufficiency of disclosure

The process of claim 1 did not encompass non-working embodiments. Document D13 did not disclose any evidence for this.

Priority

The claimed process was at least entitled to the second priority right originating from European application EP 07002571.3 filed on 6 February 2007. Thus, documents D25 and D31 were not prior art (Article 54 EPC).

Novelty

Document D2 disclosed a perfusion culturing of cells for the production of an antibody. The process used *inter alia* a hollow fiber module immersed in the cell culture for a nutrient/waste exchange. The culture medium in document D2 did not flow in a substantially parallel/tangential direction over the filter surface, because the flow encompassed perpendicular and turbulent elements.

Document D13 disclosed a membrane filter, not a hollow fiber filter.

Documents D15 and D16 disclosed a filtration system that was based on dialysis, i.e. a system that was fundamentally different from the claimed filtration resulting from a substantially parallel flow of the culture medium over the filter surface that resulted in a liquid outflow. A liquid outflow as referred to in claim 1 was the necessary result of a pressure gradient across the membrane. Dialysis led not to a net liquid outflow over the membrane. Substances passed the membrane only if a concentration gradient was present. The use of pumps as disclosed in documents D15 and D16 did not necessarily cause a pressure gradient across the membrane because they could be synchronised.

Inventive step

Document D20 represented the closest prior art. The claimed process differed from the process of document D20 solely in the pore size of the hollow fiber filter. While the filter in the claimed process retained the cells and the recombinant protein within the cell culture, the pore size in document D20 was so large that antibodies and some cells passed through the filter. The product resulting from the claimed process was more concentrated compared to the process of document D20 (11.1 g/l IgG see Example 2 of the patent vs 0.47 g/l of IgG in document D20). The technical problem to be solved was thus the provision of an improved process for the culturing of suspended cells producing a desired biological substance in a cell medium. This problem was solved by using a hollow fiber filter with a pore size that separated the recombinant

protein from substances having a lower molecular weight already during culturing.

The processes reported in documents D2 and D21 did not represent the closest prior art. Document D2 used a different filtration system. Due to the rotational movement of the cell culture relative to an immobilised filter, the cell culture did not flow substantially parallel to the filter surface but in a perpendicular and turbulent manner. Document D21 likewise used a different filtration system. The cells and antibodies were retained in the reactor with a so called dead-end filtration, i.e. a filtration that resulted from a perpendicular flow of the culture medium relative to the filter surface.

Document D5 disclosed a perfusion culturing of suspended cells in serum-containing medium for the production of an antibody. The process used an external membrane filter with a pore size of 0.22 or 0.65 microns (see abstract, page 157, column 1, fourth paragraph and Figure 2). The serum proteins of the medium caused a so called gel polarisation of the filter membrane. This reduced the filter's effective pore size to an extent resembling an ultrafiltration membrane without disclosing it's actual pore size. No indications were derivable from document D5 whether this effect was reproducible for other filter types, in particular hollow fibers. The claimed process differed from the process of document D5 in the use of a different filter with a different pore size.

Document D13 disclosed a perfusion culturing of suspended cells producing an antibody in a reactor with a cell settling zone (see abstract and Figure 1). This settling zone prevented the circulation of the majority

of cells over an external membrane filter. The document mentioned that in preliminary experiments the out flow of cells from the reactor's cell culture caused filter clogging. Thus, the culture supernatant of document D13 differed from the cell culture in claim 1 in not comprising substantially all cells. The filter clogging problem was solved in a second experimental set up by using an additional cell trap. The combined use of a settling zone and a cell trap prevented the circulation of cells over the membrane filter.

XIV. The appellant requested that the decision under appeal be set aside and the patent be revoked. Furthermore, the appellant requested that auxiliary requests 1 to 6 not be admitted into the appeal proceedings.

XV. The respondent requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request, filed as auxiliary request 1 under cover of a letter dated 16 October 2019. Furthermore, the respondent requested not to admit documents D61 and D62 into the proceedings as well as a new line of argument of the appellant under insufficiency of disclosure based on document D7.

Reasons for the Decision

Admission into the appeal proceedings of the main request (submitted as auxiliary request 1), of documents D22, D61 and D62, and a new argument under insufficiency of disclosure based on document D7

1. According to the established case law, the function of an appeal is to give a judicial decision upon the correctness of a separate earlier decision taken by an examining or opposition division. The admission

(consideration) of new claim requests or lines of argument into the appeal proceedings is at the board's discretion (Article 114(2) EPC and Articles 12(4) and 13(1) RPBA 2007; see (see Case Law of the Boards of Appeal of the EPO, 10th edition 2022 ("Case Law"), V.A. 1, 1281 and V.A.4, 1368).

2. The main request (former auxiliary request 1) was filed on 16 October 2019 in reply to the appeal. Accordingly, the requirements of Article 12(4) RPBA 2007 apply (see also Article 25(2) RPA 2020). The principal criterion on admission under Article 12(4) RPBA 2007 is whether a claim request could have been filed earlier.

3. The appellant objected to the admission of the auxiliary requests into the appeal proceedings, because they lacked convergence.

4. In reply to the appeal, the respondent filed *inter alia* auxiliary request 1 (i.e. the main request in the appeal proceedings), and explained the reasons for its filing and the amendments introduced therein. This request is identical to auxiliary request IV already filed at first instance. None of the auxiliary requests submitted during the first instance proceedings were examined because the opposition division decided in the patent proprietor/respondent's favour on the basis of the main request.

5. The issue of admission concerns the main request only. Already for this reason, lack of convergence cannot apply. The feature "*hollow fiber*" was introduced into claim 1 to address an objection under lack of novelty raised by the appellant against the claims as granted. Thus, the main request complies with Rule 80 EPC.

6. In light of these considerations, the board considers that the main request represents an adequate reaction of the respondent to the course of the appeal proceedings.

7. The main request is thus admitted into the appeal proceedings (Article 12(4) RPBA 2007).

8. Document D22 was not admitted by the opposition division into the proceedings because it was held not to be publicly available (see decision under appeal, point 7.4).

8.1 The board shares this view. Based on a balance of probabilities it is not convincing that the presentation of document D22 was publicly available. Document D22's front page states explicitly that the presentation is confidential. This statement contradicts Mr. Bonham-Carter's declaration (see D44), who is an employee of an opposing party (opponent 03), that the presentation was given to the public in the years 2002-2004 under no confidentiality constraints.

8.2 Furthermore, other evidence supporting Mr. Bonham-Carter, for example, from at least one attendee of the sales presentations, is not available. Thus document D22 is not prior art under Article 54(2) EPC, and will be disregarded in these proceedings.

9. As regards the admission of documents D61 and D62, the board sees no reason why the appellant could not have submitted these documents already in the first instance proceedings, since sufficiency of disclosure and lack of inventive step were grounds of opposition since the onset of the proceedings. Furthermore, as set out above, the main request has been submitted already

during the first instance proceedings. Further, the claimed process of the main request is not limited by a membrane with a defined cut off size. This raises doubts about the *prima facie* relevance of these two documents. Documents D61 and D62 are thus not admitted into the proceedings (Article 12(4) RPBA 2007).

10. As regards the admission of the appellant's line of argument under insufficiency based on document D7, this argument was not submitted during the first instance proceedings too. As mentioned above, sufficiency of disclosure was a ground for opposition from the onset of the proceedings. Therefore, this argument could, and should have been submitted by the appellant in the first instance proceedings. This line of argument is therefore not admitted (Article 12(4) RPBA 2007).

Main request

Claim construction - claim 1

11. In the board's understanding the process of claim 1 aims at the manufacturing of recombinant proteins in general produced by suspended eukaryotic cells cultured in serum-free medium in a reactor.

12. This aim must be achieved by (i) feeding at least one medium component into the cell culture, and (ii) by circulating the culture medium over a hollow fiber filter in a flow substantially parallel to the filter's surface resulting in a liquid outflow and a flow, the contents of which are kept in, or fed back into the reactor. Furthermore, at least one medium component is constantly replaced.

13. This type of process is known as cell perfusion culturing, wherein waste products are constantly removed while at least one nutrient is replenished (see e.g. document D7, page 62, column 1, second paragraph).

14. The hollow fiber filter mentioned in claim 1 is functionally, not structurally, characterised. The filter's pore size must be suitable to separate a recombinant protein from lower molecular weight components. The liquid outflow of the reactor consists "*essentially*" of components having a lower molecular weight than the recombinant protein, which implies that a small percentage of the desired protein leaks through the filter and is discarded in the outflow. Furthermore, the filter must either retain the recombinant protein in the culture medium in the reactor, or means must be used so that the protein can be fed back to the reactor subsequently.

15. Since the hollow fiber filter in claim 1 is not defined except that it must be suitable for the purposes indicated above, the claim encompasses the use of these filters at any pore size. Furthermore, the retaining of the recombinant protein in the medium requires that the filter must be located (immersed) in the cell culture inside the reactor. If the recombinant protein is subsequently fed back to the reactor, the filter must be located outside of the reactor.

16. The terms "*eukaryotic cell*", "*suspension*", and "*reactor*" are also not defined in claim 1. Hence, the claim encompasses the use of any eukaryotic cell grown in solution, i.e. not adhered to surfaces, and any reactor design/size suitable for producing recombinant proteins from suspended cells. Any reactor design includes the use of reactors with "*pre-filters*" in the

claimed process to prevent a premature filter clogging by cells, as for example, mentioned in paragraph [0027] of the patent. Likewise the "*cell-trap*" and "*settling zone*" disclosed in document D13 represent pre-filters (see page 173, column 1, third paragraph, and page 176, column 1, third paragraph).

17. Furthermore, the term "*cell culture*" in claim 1 is not defined, except for containing at least eukaryotic cells, a recombinant protein, and serum-free medium as minimum constituents. The presence of other ingredients is not excluded therefrom due to the use of the comprising language. Thus the claim encompasses the use of any such cell culture, irrespective of the cells' density/viability, and the protein's concentration.

18. The claim further requires that "*the cell culture... is circulated over a filter in a flow substantially parallel to a surface of said filter*".

18.1 Since the term "*cell culture*" is not defined, except for its minimal constituents (see above), claim 1 encompasses the circulation of the cell culture as a whole, or any portion thereof that comprises at least these three constituents in any concentration. Therefore claim 1 does not require that the whole cell culture is circulated over the filter.

18.2 A further issue in this context is how the circulation of culture medium *"over a filter in a flow substantially parallel to a surface of said filter resulting in a liquid outflow and a flow which contents are kept in or fed back into the reactor*" must be construed. Either narrowly in that it necessarily implies a tangential flow filtration (TFF), i.e. a specific type of filtration (see paragraph [0027] of

the patent), or broader in that it relates to any filtration resulting from the circulation of a parallel culture flow over the filter surface.

18.3 In the board's opinion, the skilled person would construe this feature as relating to any filtration resulting from a substantially parallel flow of the culture medium over the filter surface. Moreover, the resulting liquid outflow necessarily implies the presence of a pressure gradient across the membrane. Contrary thereto dialysis-based filtrations have no net liquid outflow, because the compound exchange across the membrane follows a concentration gradient (see document D9, page 1487, point 66).

18.4 Furthermore, since the direction of the substantially parallel flow of the culture medium is not defined in claim 1, the process encompasses a unidirectional as well as a bidirectional (alternating) flow of the cell culture relative to the filter surface.

Added subject-matter - claim 1

19. In the following all references to the application as filed refer to the earlier patent application (WO 2008/006494).

20. The appellant submitted two lines of argument under added subject-matter against the process of claim 1. The application as filed provided no basis (i) for the combination of features in claim 1, and (ii) for replacing the term "*essentially only contains*" by "*consists essentially of*" in claim 1.

21. As regards the first line of argument, the appellant submitted that the claimed process added subject-matter

because the features "*eukaryotic cells*", "hollow fiber *filter*", "*substantially parallel*", "*recombinant protein*", and "*serum-free*" in claim 1 were selected from different lists, while the combination of these features was not disclosed in individualised form in the application as filed.

21.1 The board does not agree.

21.2 The process as defined in claims 1, 4 and 5 as filed discloses a culturing of suspended cells for the production of a biological substance, which is preferably a "*recombinant protein*". Moreover, the process uses preferably a "*hollow fiber filter*" as separation system. Furthermore, page 10, lines 29 to 31 of the application as filed discloses a biological substance which "*may be produced by the cells, for example by expressing a (recombinant) gene coding therefore are for example (recombinant) proteins*".

21.3 As regards the producer cell, the application as filed discloses only two generic cell types, one "*eukaryotic*" and the other "*prokaryotic*" (see page 3, lines 5 to 7, and 11). The use of one of two equal alternatives represents no selection from a list.

21.4 The application as filed further discloses that the "*circulation of the cell culture over a filter may be a flow substantially perpendicular with respect to the filter surface, also known as dead-end flow or a flow substantially parallel to the filter surface, also known as tangential flow*" (see page 6, lines 24 to 26). Thus two alternatives are disclosed for circulating a cell culture over the filter, a perpendicular flow or a parallel, i.e. tangential flow. The use of one of two alternatives results not in a selection from a list.

21.5 Lastly, page 13, lines 4 to 7 of the application as filed states that for "*production of biological substances according to the invention, in particular if the biological substances are to be used as an active ingredient in pharmaceutical preparations, serum free media are preferred to media containing a serum source*". This passage provides a pointer to the skilled person that the use of serum-free media for the production of biological substances is preferred.

22. As regards the second line of argument, the appellant submitted that the replacement of the expression "*the liquid outflow will essentially only contain*" as disclosed on page 5, line 33 and 34 of the application as filed by "*the liquid outflow consists essentially of*" in claim 1 added subject-matter because both expressions were not equivalent.

23. The board agrees with the opposition division's finding in the decision under appeal that the expression "*the liquid outflow consists essentially of*" in claim 1 finds a basis on page 5, line 33 to page 6, line 1 of the application as filed which states "*Usually, the liquid outflow will essentially only contain components having a molecular weight lower than that of the biological substance. Essentially all cells and essentially all biological substance are therefore usually retained in the reactor*" (emphasis added).

24. The passage on page 5 indicated above as well as claim 1 define "*essentially*" what the outflow contains. Thus and contrary to the appellant's view, the use of essentially in combination with "*consists*" in claim 1 has the same meaning when compared to the respective

expression "*essentially only contain*" on page 5, lines 33 and 34 of the application as filed.

25. The feature "*mammalian cells*" in claim 3 is literally disclosed in claim 3, and on page 3, lines 15 and 16 of the application as filed, which states that these cells are used "*in particular*".

26. The various cell lines mentioned in claim 4 are literally disclosed on page 3, lines 17 to 19 of the application as filed.

27. The two specific cells mentioned in claims 5 and 6 are mentioned on page 3, line 20, and in claim 3 of the application as filed.

28. The feature "*antibody*" of claim 7 is mentioned in claim 4 as filed.

29. Consequently, the application as filed provides a basis for the subject-matter of the main request. Accordingly, the main request complies with the requirements of Articles 76(1) and 123(2) EPC.

Sufficiency of disclosure

30. The appellant submitted that the claimed method encompassed non-working embodiments because processes reported in the prior art, although comprising all the features of claim 1, suffered from filter clogging due to the presence of cells (see document D13, Figure 1 on page 172, and page 176, column 1, third paragraph).

31. According to decision G 1/03 (OJ EPO 2004, 413), if there is a lack of reproducibility of the claimed invention (i.e. a failure of the claimed features to

deliver an aimed for effect), this may be relevant under the requirements of sufficiency of disclosure or inventive step. If the technical effect is expressed in the claim, there is a lack of sufficiency of disclosure. Otherwise, there is a problem of inventive step (cf. G 1/03, point 2.5.2 of the Reasons).

32. Claim 1 mentions as technical effect the use of a hollow fiber filter that results in a "*liquid outflow and a flow which contents are kept in or fed back into the reactor*" resulting from the circulation of culture medium that flows substantially parallel to the filter surface. Furthermore, the filter must be "*suitable*" for separating the recombinant protein from substances with a lower molecular weight. Accordingly, it must be assessed under Article 83 EPC whether or not the features in claim 1 achieve these effects, i.e. (i) a retention of recombinant proteins in the medium, and (ii) a separation and removal of substances having a molecular weight lower than the recombinant protein in the liquid outflow.

33. The board has no doubts that hollow fiber filters as defined in claim 1 are suitable for these purposes. This has also not been contested.

34. Rather the appellant's objection is that the filter because of filter clogging, while not in the beginning but over time, looses it's ability to separate/remove substances with a molecular weight lower than that of the recombinant protein from the cell culture.

34.1 The claimed process does not define a cultivation time for the cells, or another end point of the process, for example, a minimum protein concentration, or a certain cell viability/density. Accordingly, clogging of the

filter over time for whatever reason is irrelevant for assessing sufficiency of disclosure in this case. The process merely requires that at least some protein is produced by cultivating eukaryotic cells in solution using a hollow fiber filter under the conditions specified in claim 1, so that the recombinant protein of interest is retained/fed back, while smaller components are removed, and at least one medium component is fed to the culture.

34.2 No arguments have been submitted, let alone evidence that the claimed process is not in principle suitable for these purposes. Document D13 provides no evidence in this respect. The document discloses that a process using a filter can be further improved by, for example, using an additional cell trap (see page 176, column 1, second and third paragraph).

35. Thus claim 1 and, hence, the main request complies with the requirements of Article 83 EPC.

Priority

36. The appellant contested *inter alia* the entitlement to the priority right originating from document EP2007005915.3 filed on 6 February 2007 ("P2 document"), for the same reasons as those submitted under added subject-matter.

37. It is uncontested that the description of the P2 document is identical to that of the patent application. Furthermore, as concluded above, the subject matter of the main request does not extend beyond the content of the patent application as filed.

38. Accordingly, and in analogy to the finding on added subject-matter, the process of claim 1 is entitled to the priority right arising from the P2 document, because the criteria for examining added subject-matter and priority entitlement are the same (see Case Law, II.D.3.1.2). The relevant filing date of the patent application is therefore 6 February 2007.

39. As a consequence, documents D25 and D31 are not prior art because they are published on 14 January 2009 and 10 May 2007, respectively, i.e. later than 6 February 2007.

Novelty

40. The appellant submitted that the process of claim 1 of auxiliary request 1 lacked novelty for the same reasons as set out for the main request, i.e. the claimed process lacked novelty over the disclosure of documents D2, D13, D15, D16, and D27 to D30.

41. Document D2 discloses a process for the production of IgG from suspended mammalian cells in a reactor. Fresh medium is pumped through a filter immersed in the cell culture inside the reactor. This filter constantly supplies the culture medium with fresh nutrients while waste products are removed therefrom. *Inter alia* a hollow fiber filter is used characterised by a MWCO preferably in the range of 3 kDa to 10 kDa (see paragraphs [0001], [0010], [0015], [0028], and [0035]).

41.1 The nutrient/waste exchange within the filter results from the circular pumping of fresh medium through the lumen side of the filter in a substantially parallel flow direction mediated by a pressure gradient (see paragraphs [0010] and [0021]). The exchange is

supported by a rotational movement of the culture medium within the reactor. An impeller forces the culture medium to flow around ("*umfließt*") the external side of the filter. This external movement of the culture medium is independent from the fresh medium's internal flow inside the filter, or the medium's alternating dead end flow in filters with a single inlet (see paragraphs [0023], [0031], and [0034]).

41.2 The appellant submitted that the rotational flow of the culture medium around the immersed filter in document D2 inevitably resulted in a substantially parallel flow of the culture medium over the filter's external surface.

41.3 The board does not agree. Rather it is inevitable that a constant rotational movement of the culture medium against an immobilised filter causes a flow which comprises perpendicular, tangential and turbulent elements over the filter's external surface. This prevents a substantially parallel flow of the culture medium over the filter surface, contrary to the requirements of the claim.

42. Document D13 discloses a perfusion culturing of suspended mouse-human hybridoma cells in serum and serum-free medium for the production of IgG (see abstract). A membrane filter cassette with a MWCO of 10 kDa is used for retaining IgG and cells within the culture medium (see Figure 1 in conjunction with page 172, column 1, first paragraph). This membrane filter is not a hollow fiber filter.

43. Documents D15 and D16 disclose a dialysis-based filtration which differs fundamentally from a

filtration using a substantially parallel flow resulting in a liquid outflow (see point 18.3, above).

43.1 The appellant submitted that the different cross-sectional areas of the lumen and shell side of the hollow fiber filter used for nutrient/waste exchange in documents D15 and D16 inevitably generated a pressure gradient which caused a cross flow of nutrients/waste products across the filter. The document disclosed therefore not a dialysis-based filtration but a filtration that fell within the scope of claim 1.

43.2 The board does not agree. Although document D15 designates the hollow fiber filter used for the nutrient/waste exchange as "*cross flow filter*", document D15 states that this filter allows the waste products to "*diffuse*" from the culture, and the nutrients to "*diffuse*" into the culture, while keeping antibodies and cells within the culture medium (see column 1, lines 20 to 40, column 2, lines 12 to 17).

43.3 Thus the nutrients/waste exchange over the filter is caused by diffusion which is a dialysis-based filtration along a concentration gradient (see column 1, lines 31 to 35, and claim 1, step (f)), and not due to a pressure gradient as required for an outflow resulting from a substantially parallel flow (see point 18.3 above, document D9, page 1487, point 66, and document D10, page 1, column 1, second paragraph, and Figure 1).). The cross flow filter in document D15 is therefore operated in a dialysis mode.

43.4 The skilled person would take this disclosure in document D15 at face value. A pressure gradient does not allow a simultaneous exchange of nutrients/waste across a filter membrane along respective concentration

gradients, but presses substances across the membrane along a pressure gradient - even against a concentration gradient. Pressure gradients within the hollow fiber filter can be avoided by synchronising the pumps. Therefore it is not inevitable that pumps in combination with different sectional areas of the lumen and shell sides of a hollow fiber filter generate pressure gradients across a membrane.

43.5 The same is true for document D16 which designates the filter consistently as "*dialyser*" (see e.g. column 7, lines 43, 46, 56, and column 8, lines 34 to 41). This is consistent with the filter's function as a dialyser since document D16 states that the exchange speed of nutrients/waste via the membrane is "*dependent upon the concentrations of the materials in question on both sides of the membrane and upon the membrane surface*" (see column 10, lines 10 to 16). Thus, the nutrients/waste exchange depends solely on a concentration gradient (i.e. a classical dialysis).

44. Documents D27 to D30 do not represent "poisonous divisionals" or a "poisonous parent" for the subject-matter claimed. In decision G 1/15 (published in OJ 2017, 82) the Enlarged Board of Appeal has ruled that partial priorities can be claimed for a claim encompassing alternative subject-matter even if the claim comprises one or more generic expressions provided that said alternative subject-matter has been disclosed for the first time, directly, or at least implicitly, unambiguously and in an enabling manner in the priority document.

45. Consequently, the process of claim 1 and, hence, the main request is novel and complies with the requirements of Article 54 EPC.

Inventive step

Closest prior art and technical problem

46. The appellant submitted that any one of documents D2, D5, D13, D15, D16, and D21 represented the closest prior art. The respondent selected document D20, while the opposition division considered that document D5 represented the closest prior art.

47. According to the case law the closest prior art must be directed to the same purpose as the claimed invention, and, as a secondary consideration, should have most of the relevant technical features in common.

48. Document D2 discloses a perfusion culturing of cells for the production of IgG that is silent on the use of serum-free medium. Moreover, the filtration of the culture medium is based on a different principle (see point 41.3 above).

49. Document D5 discloses a perfusion culturing of cells producing an antibody in serum containing medium (see abstract, page 156, column 1, second paragraph, and Figure 2). The exchange between nutrients and waste products in the process is achieved in a filter unit using TFF (see Figure 2, Legend). The filter is "*equipped with either a 0.22 or 0.65 micron Durapore (millipore DVLP) membrane*" (see page 157, column 1, fourth paragraph), for which the effective MWCO is not disclosed. Document D5 states in this context on page 163, column 1, starting at line 7: "*Both the 0.65 micron or 0.20 micron microporous membranes exhibited gel polarization by medium derived serum proteins resulting in retention of MAB similar to an ultrafilter*

[5]" (emphasis added). Reference "*[5]*" is identical to document D13 in these proceedings. In other words, the gel polarised filter of document D5 separates the antibody from substances having a lower molecular weight. A hollow fiber filter is not mentioned in document D5.

49.1 It follows from the passage on page 163 of document D5 indicated above that the use of serum containing medium is essential for the disclosed process. Solely the serum proteins in the medium reduce the effective pore size of the microporous filter to a size suitable for retaining the antibodies in the culture medium.

49.2 In view thereof it is not convincing that the skilled person would have considered document D5 to represent the closest prior art for the claimed process wherein cells are cultured in serum-free medium. The filtration system in claim 1 in the absence of serum proteins is incompatible with the gel polarisation-based system of document D5. In these circumstances, the skilled person would have rather considered documents disclosing cell culturing in serum-free medium as the appropriate closest prior art, for example, document D13 (see below).

50. Document D13 discloses a perfusion process for producing IgG in suspended mammalian cells in serum-free medium (see point 42 above). This document is silent on a hollow fiber filter but uses instead a Millipore Pellicon membrane filter (see page 172, column 1, first paragraph).

51. The appellant's inventive step argumentation based on documents D15 and D16 has not been substantiated. In view of the board's finding on novelty outlined above

(see point 43), both documents disclose a filtration system that is fundamentally different from the claimed one (dialysis instead of pressure gradient dependent filtration). Thus documents D15 and D16 do not qualify as closest prior art.

52. Document D20 discloses a process for a perfusion culturing of suspended eukaryotic cells producing recombinant proteins in serum-free medium, wherein the cell culture is circulated over a hollow fiber filter in an ATF mode. The resulting liquid outflow has a lower cell density than the cell culture, i.e. a certain percentage of cells leaks through the filter (see page 1, lines 6 to 11, page 3, line 33 to page 4, line 9, page 7, lines 30 and 31, and claim 1).

52.1 The hollow fiber filter has a pore size with a MWCO of "*0.2 micron*", i.e. 0.2 µm (see page 8, lines 11 to 16, page 10, line 20), which explains the cell leakage. The process produces preferably monoclonal antibodies which are, due to the large filter pore size, constantly removed from the cell culture (see page 9, lines 14, 20, 26, 29 and 31, Examples 1 and 2, page 7, line 30, and page 11, line 14, and claim 14).

52.2 The claimed process therefore differs fundamentally from the process of document D20. A filter with a pore size that is suitable to separate the recombinant proteins from lower molecular weight substances retains, and thereby concentrates these proteins including the producer cells within the culture medium instead of constantly removing them therefrom.

53. Document D21 discloses a perfusion culture of a mouse hybridoma cell that produces IgG (see abstract, and page 36, column 1, penultimate paragraph). To retain

the cells and the antibody inside the reactor, a membrane with a MWCO of 10 kDa was fixed to the bottom of the reactor resulting in a so called "dead-end filtration" (see page 36, column 2, second paragraph). The cultivation was performed in serum-free medium (see page 36, column 2, last paragraph). A hollow fiber filter is not disclosed. The claimed process therefore differs fundamentally from the process of document D21, since a circulation based on a substantial parallel flow is used for filtering, instead of a perpendicular flow causing a dead-end filtration.

54. In summary, all documents referred to above are directed to the same purpose as the claimed process, namely the production of recombinant proteins in suspended eukaryotic cells. However, for the reasons indicated above documents D2, D5, D15, D16, D20 and D21 disclose fundamentally different filtration systems in their processes which makes them unsuitable as closest prior art. For this reason the appellant's, and the respondent's problem-solution approach arguments provided for these documents are not convincing, and will be disregarded. Accordingly, document D13 represents the closest prior art.

55. The respondent submitted that the difference between the use of a hollow fiber filter used in the claimed process and a membrane filter disclosed in document D13 resulted in the production of recombinant proteins, in particular IgG, with an increased yield. This was shown in Example 2 of the patent.

55.1 According to the established case law, alleged advantages to which the patent proprietor merely refers, without offering sufficient evidence to support the comparison with the closest prior art, cannot be

taken into consideration in determining the problem underlying the invention and therefore in assessing inventive step (see Case Law, I.D.4.3.1).

55.2 No comparative experimental data of the claimed process using a hollow fiber filter versus a membrane filter have been provided by the respondent. Accordingly, the Examples in the patent are not suitable to support an increased IgG yield across the whole breadth of the claim.

56. The technical problem to be solved is thus the provision of an alternative process of culturing suspended eukaryotic cells for the production of a recombinant protein.

57. In view of the working example of the patent, the board is convinced that the process of claim 1 solves this problem.

Obviousness

58. It remains to be assessed whether or not the skilled person, starting from document D13 and facing the problem defined above, would have arrived at the process of claim 1 in an obvious manner.

59. The appellant submitted that the claimed method was obvious for the skilled person in light of document D13's teaching alone since it merely required the replacement of a membrane filter by a hollow fiber filter, or by combining document D13 with a document that discloses the ATF technology, for example, document D17.

60. The board does not agree. Starting from the first experimental set-up reported in document D13, the skilled person would not have merely replaced the membrane filter with a hollow fiber filter, since he or she would have expected that the indicated filter clogging problem remained the same irrespective of the type of filter used. In this situation, the skilled person would have followed the explicit teaching of document D13 for solving the clogging problem by using an additional cell trap. Since the filter clogging problem is solved, the skilled person has no reason to consider alternatives for solving the clogging problem, let alone the use of a hollow fiber filter together with ATF.

60.1 Starting from the second experimental set-up disclosed in document D13, i.e. the use of a settling zone together with a cell trap, the board agrees with the appellant that the exchange of a membrane filter by a hollow fiber filter is obvious because both are equivalent filter alternatives in cell culturing processes.

60.2 However, the skilled person would not necessarily arrive at a process that falls within the scope of claim 1. The use of the settling zone combined with a cell trap in the process of document D13 might be so efficient at removing cells from the cell culture supernatant circulating over the filter, that the supernatant does not necessarily contain cells, contrary to the requirements of claim 1 (see point 17, above).

61. In light of these considerations, the process of claim 1 is not obvious over the prior art. The main request therefore involves an inventive step (Article 56 EPC).

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent with the following claims and a description to be adapted:
   Claims: No. 1 to 11 of the main request, filed as auxiliary request 1 under cover of a letter dated 16 October 2019.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated