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Datasheet for the decision of 18 January 2019

Case Number: T 1790/13 - 3.3.08

Application Number: 04768294.3

Publication Number: 1664278

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Language of the proceedings: EN

Title of invention:
Methods for producing recombinant proteins

Patent Proprietor:
UCB Pharma, S.A.

Opponents:
Lonza AG
Olswang LLP

Headword:
Growth rates control partitioning of recombinant proteins between periplasm in E. coli and supernatant/UCB PHARMA

Relevant legal provisions:
EPC Art. 100(a), 100(b), 100(c), 54, 56
EPC R. 115(2)
RPBA Art. 12(4), 15(3)

Keyword:
Claims as granted - requirements of the EPC met (yes)

Decisions cited:
G 0001/03, T 0848/93

Catchword:

Europäisches Patentamt
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Beschwerdekammern

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Case Number: T 1790/13 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 18 January 2019

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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted on 20 June 2013 rejecting the opposition filed against European patent No. 1664278 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman B. Stolz
Members: M. Montrone
R. Winkelhofer

Summary of Facts and Submissions

I. An appeal was lodged by opponent 02 (hereinafter "appellant") against the decision of an opposition division to reject the oppositions against the European patent No. 1 664 278, which was filed as an international application and published as WO 2005/024000 (hereinafter the "patent application"). The patent has the title "Methods for producing recombinant proteins".

II. In the opposition proceedings, the grounds for opposition according to Article 100(a) EPC (lack of novelty and lack of inventive step), and Articles 100(b) and 100(c) EPC were evoked. The opposition division took the view that the patent as granted complied with the provisions of the EPC and rejected the opposition.

III. With its statement of grounds of appeal, the appellant submitted arguments as to why the opposition division erred in its decision that the patent as granted complied with the requirements of the EPC, in particular with regard to added subject-matter, sufficiency of disclosure, novelty and inventive step. In support of its case, the appellant filed new documents D24 to D28 and an Annex of eight tables (document D29) with data relating either to various Figures and Tables in the patent or to Figures disclosed in documents D16 or D23.

IV. Opponent 01, as a party as of right, remained silent throughout the appeal proceedings.

V. The patent proprietor (hereinafter "respondent") maintained that all patentability requirements were met.

VI. The parties were summoned to oral proceedings. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's provisional, non-binding opinion on some of the legal and substantive matters of the case.

VII. In reply thereto, the appellant, without filing any substantive submissions, informed the board that it would not be attending the oral proceedings. The respondent replied to the board's communication by filing substantive submissions concerning the admission into the appeal proceedings of (i) documents D24 to D28; (ii) Tables 1 to 8 of the Annex (document D29) including lines of arguments based thereon; and (iii) the new line of argumentation under Article 100(c) EPC. Further submissions of the respondent concerned Article 54 EPC. In addition, the respondent informed the board that it would also not be attending the oral proceedings.

VIII. Oral proceedings were held on 18 January 2019 in the absence of all parties.

IX. Claim 1 as granted reads:

"1. A method for selecting a growth rate for controlling the partitioning of a recombinant protein between the supernatant and the periplasm in *E.coli* host cell cultures such that the partitioning of the recombinant protein is most suited to the primary recovery of the recombinant protein, wherein expression

of the recombinant protein by said cells is under the control of an inducible system, which method comprises:

a) providing an *E.coli* host cell culture
b) changing the growth rate of the *E.coli* host cells such that the growth rate is in the range of 0.0005 to 0.04/h.
c) inducing expression of the recombinant protein
wherein steps (b) and (c) can be performed in any order or simultaneously; and subsequently
d) determining the yield of recombinant protein in the culture supernatant and the *E.coli* host cell periplasm
e) comparing the yield determined in step (d) with the yield determined when at least one other growth rate has been used in step (b)
f) selecting a growth rate from the comparison made in step (e) in which the partitioning of the recombinant protein between the supernatant and the periplasm is most suited to the primary recovery of the recombinant protein".

Dependent claims 2 to 20 define specific embodiments of the method of claim 1.

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

D2: A. Shokri *et al.*, Applied Microbiology Biotechnology, 2002, Vol.58: pages 386-392;

D3: A. Shokri and G. Larsson, Microbial Cell Factories, 2004, Vol.3: page 9;

D12: C.S. Shin *et al.*, Applied Microbiology Biotechnology, 1998, Vol.49: pages 364-370;

D14: J. Fu *et al.*, Biotechnology and Bioengineering, 1993, Vol.41: pages 937-946;

D16: T. Shibui and K. Nagahari, Applied Microbiology Biotechnology, 1992, Vol.37: pages 352-357;

D23: G. Miksch *et al.*, Archive Microbiology, 1997, Vol.167: pages 143-150;

D24: S.Y. Lee, TIBTECH, 1996, Vol. 14: pages 98-105;

D25: S.C. Makrides, Microbiological Reviews, 1996, Vol.60: pages 512-538;

D26: D.P. Humphreys *et al.*, Protein Expression and Purification, 2002, Vol.26: pages 309-320;

D27: "Fermentation Microbiology and Biotechnology", Ed. E.M.T. El-Mansi and C.F.A. Bryce, 1999, Chapter 4: pages 69-119;

D28: A. Minsky *et al.*, Proceedings of the National Academy of Sciences, 1986, Vol.83: pages 4180-4184;

D29: Annex of Tables 1 to 8 filed with the appellant's statement of grounds of appeal.

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

Admission of documents D24 to D28 into the appeal proceedings

The admission of documents D24 to D26 was justified since they were already mentioned in the patent and reflected the background knowledge of the respondent when filing the patent application. Furthermore, the opposition division did not take into consideration the full background/common general knowledge of the skilled person when arriving at its decision, in particular in the field of recombinant protein expression in *E. coli* for which many textbooks existed.

The disclosure in documents D27 and D28 addressed the opposition division's finding in the decision under appeal that document D14 did not directly and unambiguously disclose (i) a growth rate in the range of 0.0005/h to 0.04/h (but rather a dilution rate), and (ii) the determination of β-lactamase's yield in the periplasm (but rather its total yield including that of the cytoplasm).

Admission into the appeal proceedings of supplementary experimental data and lines of arguments based thereon in relation to novelty (Articles 100(a) and 54 EPC)

The supplementary data in Tables 7 to 8 of the Annex addressed the opposition division's finding that claim 1 as granted was novel over the disclosure in documents D16 and D23. The data in both tables provided support that the documents disclosed a "*change*" of the growth rate and a determination of the protein's yield in the supernatant and the periplasm at growth rates falling within the range of 0.0005/h to 0.04/h recited in step b) of claim 1.

Admission into the appeal proceedings of supplementary experimental data and lines of arguments based thereon

in relation to sufficiency of disclosure (Article 100(b) EPC)

The supplementary data in Tables 1 to 6 of the Annex (document D29) provided support that the patent insufficiently disclosed the claimed invention. The growth rate referred to in claim 1 had to be assessed at the time point of harvest of the protein in the supernatant and the periplasm. However, the patent disclosed in Example 1 that the growth rate and the yield were determined at different time points (see Figures 1 and 2 for the glycerol feed and Figures 5 and 6 for the phosphate feed).

Furthermore, the average or specific growth rates determined at glycerol feed rates in the range of 0.5 to 2.56 ml/h failed to show an effect on the partitioning of the protein into the periplasm as disclosed in columns (i) and (ii) of Table 1 of the Annex. Also the data disclosed in Example 2 of the patent failed to demonstrate that the phosphate feed had an impact on the growth rate, or that the growth rate controlled the protein partition (see Tables 3 and 4 of the Annex).

Further Example 1 and Figures 1 to 3 of the patent showed that data concerning the pre-induction growth rates during the glycerol feed varied to a large extent and were thus not suitable in supporting the enablement of the method over the whole breadth of the claim. The same applied to the data disclosed in the patent with regard to the phosphate feed (see Figure 4). Also the culture conditions reported in Example 2 were prone to operator based variations.

Lastly, claim 1 lacked essential technical features since it did not recite, for example, fed-batch culture conditions, an exponential growth of the cells prior to protein induction, or a method for growth rate determination.

Admission of a new line of argumentation with regard to Article 100(c) EPC

Claim 1 comprised added subject-matter since it was directed to a "method for selecting a growth rate for controlling the partitioning of a recombinant protein between the supernatant and the periplasm in *E. coli*", while the patent application was silent on selecting a growth rate in the context of the claimed method.

Main request (claims as granted)

Novelty (Article 100 in conjunction with Article 54 EPC)

The subject-matter of claim 1 lacked novelty over the disclosure in documents D14, D16 and D23.

Document D14 disclosed a method for excreting a β-lactamase protein from an *E. coli* host preferentially into the supernatant using a two-stage chemostat (see abstract and page 937, column 2, first paragraph). The document mentioned that the protein was induced only in the second stage after the culture conditions were in a steady state. In these circumstances the dilution rate corresponded to the growth rate, and the dilution rate had changed compared to the first stage. The document further showed dilution rates falling within the range of 0.0005 to 0.04/h recited in step b) of claim 1 (see abstract, page 939, column 1, last paragraph to column

2, second paragraph). Furthermore, the document reported that the protein yield was determined in the supernatant and the periplasm of the *E. coli* host cells grown at different rates, for example, at 0.03/h (see Figure 2), which provided the highest protein secretion into the supernatant (see abstract).

Document D16 disclosed an *E. coli* host cell that, after adding the inducer IPTG, efficiently excreted a recombinant Fab fragment into the supernatant (see abstract). The document further disclosed that the growth rates of the cells changed over time due to the consumption of nutrients in the medium (see Figure 2) to values that fell within the range recited in claim 1. Such a natural change in growth rates was encompassed by the feature "*changing the growth rate*" as recited in step b) of claim 1. The document also reported an accumulation of the recombinant protein during a 8-10h post-induction period in the periplasm reaching a maximum after 10h. Then a decrease from the periplasm was observed during the period of 10-22h post-induction, accompanied by a steady increase in the culture medium (see page 354, column 2, fifth paragraph to page 355, column 1, first paragraph). Since the Fab fragment concentration was determined in the periplasm and the medium during the 8-22h post-induction period, it's yield in these two compartments was compared, the skilled person could have selected growth rates according to a desired partition ratio.

Document D23 disclosed an *E. coli* host cell expressing β-glucanase under the control of a growth phase-dependent promoter at the onset of the stationary phase only, which concomitantly induced the protein's secretion into the supernatant (see abstract, page 144, column 2, first paragraph, page 146, column 2, second

paragraph, Figure 4B). Figure 4A disclosed growth rates which fell into the range of growth rates recited in step b) of claim 1, and that the yield of the protein was determined in the periplasm and the supernatant at different growth phases (see page 147, column 2, last paragraph).

Inventive step (Article 100 in conjunction with Article 56 EPC)

Documents D12, D2, or D3 represented the closest prior art for the method of claim 1.

An objective underlying the patent was *inter alia* to improve the efficiency of the primary recovery of recombinant proteins, and hence, the protein's yield and quality (see paragraph [0007]).

The disclosure in document D12 related to the same objective (see abstract). Figures 2a, b of document D12 disclosed four fed-batch fermentations, each characterised by two pre-induction growth rates (named "μ_{AG}" and "μ_{BG}"), and two post-induction (production) growth rates (named "μ_{AP}" and "μ_{BP}"), wherein the pre-induction growth rates were always higher than those of the post-induction phase, i.e. they changed. Furthermore, Figures 2a, B and 2b, B mentioned growth rates of "$0.023h^{-1}$" and "$0.015h^{-1}$", respectively, that both fell within the range of 0.0005 to 0.04/h recited in claim 1. The method disclosed in document D12 differed from that of claim 1 in that it was silent on determining the yield of the recombinant protein in the periplasm and the supernatant. However, the directed expression of a recombinant protein into the cytoplasm, the periplasm, or the supernatant constituted mere alternatives that were standard practise of the skilled

person. Hence, the claimed method lacked an inventive step.

Document D2 related to an objective similar to that of the patent, since it disclosed *inter alia* that growth rates had an effect on the periplasmic partition of a recombinant protein in *E. coli*, without causing cell lysis. The document mentioned that the leakage of proteins from the periplasm into the medium was controlled by various mechanisms (see page 367, column 2, first paragraph). It further disclosed that the yield of a constitutively expressed recombinant protein was determined in the periplasm and the supernatant of the *E. coli* cells (see Figure 6). A dilution rate of 0.05/h (see Figures 1, 2A), i.e. a value very close to the 0.04/h defining the upper limit of the range recited in claim 1, was highlighted for a preferred protein's partition into the periplasm (see Figure 6C), because it caused a low protein leakage into the supernatant and a cell lysis of 4% only. Moreover, the dilution rate corresponded to the growth rate, since the cells were cultivated under steady state conditions (see page 387, column 1, third paragraph).

The method disclosed in document D2 differed from the claimed method in that the growth rate was 0.05/h, while the claimed growth rates were in the range of 0.005-0.04/h, and in that document D2 was silent on an induction step for the protein expression.

There was no disclosure in the patent that the small difference of 0.01/h in growth rate (0.05/h-0.04/h) was associated with a technical effect. Moreover, it belonged to the common general knowledge of the skilled person that in the production phase of a recombinant protein, the growth rate was preferably low (i.e. 0.05/

h) to achieve a high protein yield (see document D2, page 391, column 2, last paragraph). Thus, a skilled person starting from the method disclosed in document D2 aiming at a partition of proteins into the periplasm was motivated to consider "*growth rates in the range of ≤0.05/h for continuous cultivation*", and "*lower rates for fed-batch fermentation*".

Likewise there was no technical effect associated with the second distinguishing feature, i.e. the induction of gene expression. This feature was known as an alternative to a constitutive gene expression and inducible promoters were generally known in the art (see paragraph [0013] of the patent). Hence, since both distinguishing features were obvious to the person skilled in the art, claim 1 contravened Article 56 EPC.

Document D3 compared the effects of continuous and fed-batch fermentations on the lipid composition of *E. coli* cell membranes. The document reported that over a tested range of growth rates of 0.05/h to 0.6/h, the membranes of cells grown under fed-batch conditions remained stiffer, the cells showed a low lysis, and recombinant protein leaked at a constant rate into the medium (see abstract). Furthermore, document D3 disclosed that growth rates affected the partition of proteins into the periplasm.

Figure 7, for example, disclosed that at a low growth rate of 0.05/h, the partition of proteins occurred into the periplasm.

The method reported in document D3 differed from that of claim 1 in using a growth rate of 0.05/h, which was above the recited range of 0.005-0.04/h, and in that it was silent on an induced protein expression. However,

as set out above with regard to document D2, these distinguishing features did not render the method of claim 1 inventive.

Furthermore, the patent did not demonstrate that growth rates in the recited range of claim 1 had any effect on protein partition in *E. coli*.

Lastly, the skilled person had to empirically test each feed rate to assess its effect on the growth rate, then to test for an effect of the growth rate on the protein's partition between the periplasm and the supernatant, and lastly to decide which growth rate was most suited for the primary recovery of the protein. Moreover, the patent was silent on any of the conditions under which these tests were to be performed, except for a specified growth rate range, which however, lacked an inventive step and was routine in the art. Furthermore, the empirical testing of conditions affecting the growth rate was common general knowledge in the art, as indicated in the patent itself (see paragraphs [0020] and [0021]). Therefore, the patent did not solve the technical problem it purported to solve. Instead, it provided an invitation to perform a research program.

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

Admission of documents D24 to D28 into the appeal proceedings

Documents D24 to D28 should not be admitted into the appeal proceedings. With regard to documents D27 and D28, the point that they sought to address in the

decision under appeal in relation to document D14 was irrelevant, since document D14 failed to disclose a protein determination in the periplasm of *E. coli*, and thus differed from the claimed method in at least one feature.

Admission into the appeal proceedings of supplementary experimental data and lines of arguments based thereon in relation to novelty (Articles 100(a) and 54 EPC)

The supplementary data shown in Tables 1 to 8 of the Annex (see document D29) should not be admitted into the appeal proceedings too.

The data in Tables 7 and 8 (see document D29) related to subjective interpretations of two growth curves shown in documents D16 and D23, and hence, lacked any resemblance with them. Thus, the data in Tables 7 and 8 failed to provide support that documents D16 and D23 directly and unambiguously disclosed the method of claim 1.

Admission into the appeal proceedings of supplementary experimental data and lines of arguments based thereon in relation to sufficiency of disclosure (Article 100(b) EPC)

Tables 1 to 6 (see document D29) and arguments based thereon should likewise not be admitted into the appeal proceedings. The submissions were unrelated to any of the appellant's submissions made in the context of Article 100(b) EPC in the first instance proceedings, and were also not linked to issues addressed in the decision under appeal. Nor have reasons been provided by the appellant why these submissions could not have been filed in the first instance proceedings.

Furthermore, the appellant had no provided any evidence of a growth rate falling within the recited range in claim 1 that was not suitable for controlling the partition of recombinant proteins between the periplasm and the supernatant. However, the burden of proof lay with the appellant, which accordingly, has not been discharged.

Admission of a new line of argumentation with regard to Article 100(c) EPC

The allegation that claim 1 comprised added subject-matter was based on new facts. Thus, this objection should not be admitted into the appeal proceedings.

Main request (claims as granted)

Novelty (Article 100 in conjunction with Article 54 EPC)

The claimed method was novel over the disclosure in document D14 at least because D14 was silent on the determination of the protein's yield in the periplasm (see step d) in claim 1). Figure 2 in that document disclosed that the concentration of β-lactamase was lower in the supernatant compared to a whole culture sample, although the majority of the enzyme was secreted into the medium. Therefore the whole culture sample comprised the β-lactamase secreted into the supernatant plus that released from the ruptured cells. In these circumstances it was irrelevant whether or not the enzyme from the ruptured cells was derived from the periplasm only or from the periplasm and the cytoplasm.

Document D16 investigated the effects of temperature and inducer concentration on the yield of a recombinant protein in *E. coli*. However, the range of growth rates recited in claim 1 was not disclosed.

Document D23 disclosed a gene named "*kil*" (encoding a bacterial release protein) under the control of a growth-phase regulated promoter and its effects on the secretion of a recombinant protein in *E. coli*. The *kil* gene promoter expression was automatically triggered at the onset of the stationary phase, which was not encompassed by the feature "inducing expression of the recombinant protein" recited in step c) of claim 1. Furthermore, the document was silent on the specific growth rate range referred to in claim 1, since it only disclosed *E. coli* cells passing through a continuous spectrum of growth rates in a growth curve.

XIII. The appellant requests that the decision under appeal be set aside and that the patent be revoked in its entirety.

XIV. The respondent requests that the appeal be dismissed. The respondent's further procedural requests needed not to be considered by the board, in view of the outcome of the proceedings.

Reasons for the Decision

1. The duly summoned parties did not attend the oral proceedings, which in accordance with Rule 115(2) EPC and Article 15(3) RPBA took place in their absence.

Article 113(1) EPC

2. In its communication pursuant to Article 15(1) RPBA, the board expressed a reasoned provisional opinion on the issues to be discussed at the oral proceedings, which included *inter alia* the issues of admission (consideration) of documents D24 to D28 (Article 12(4) RPBA); the admission of supplementary experimental data shown in Tables 1 to 8 of the Annex (see document D29), including arguments under sufficiency of disclosure and novelty based thereon (Article 12(4) RPBA); the admission of an objection under added subject-matter (Article 100(c) EPC) against claim 1 as granted (Article 12(4) RPBA); and novelty of the subject-matter of claim 1 as granted vis-à-vis the disclosure of documents D14, D16 and D23 (Article 100(a) in conjunction with Article 54 EPC).

3. In reply to the board's communication, the appellant did not submit any substantive arguments in relation to the issues mentioned therein. Moreover, by not attending the oral proceedings, the appellant decided not to avail itself of another opportunity to orally address or comment on these issues.

4. The present decision is based on the same grounds, arguments and evidence on which the board's provisional opinion was based, including those of the written submission filed by the respondent in reply to the board's communication and the issues discussed in the decision under appeal.

Admission of documents D24 to D28 into the appeal proceedings (Article 12(4) RPBA)

5. The appellant submitted documents D24 to D28 with its statement of grounds of appeal.

6. Documents D24 to D26 are mentioned in the patent and may thus have been known to the appellant. In its communication in preparation of the oral proceedings, the board observed that the mentioning of documents D24 to D26 in the patent did not necessarily mean that they are on file and have to be considered at any point of time in opposition or appeal proceedings. The board also saw no reasons that might have prevented the appellant from filing these documents - if relevant at all - in the first instance proceedings.

7. With regard to documents D27 and D28, the board, in view of the respondent's submissions in response to the board's communication, considered the disclosure of these documents no longer relevant for interpreting the disclosure of document D14 in the assessment of novelty of the method of claim 1 (see point 34.6 below).

8. In these circumstances, in exercise of the board's discretion according to Article 12(4) RPBA documents D24 to D28 are not to be considered in the appeal proceedings.

Admission into the appeal proceedings (Article 12(4) RPBA) of supplementary experimental data shown in Tables 7 and 8 of the Annex to the grounds of appeal (document D29) and lines of arguments based thereon in relation to novelty (Article 100(a) in conjunction with Article 54 EPC)

9. The experimental data shown in Tables 7 and 8 (see document D29) and lines of arguments based thereon in

relation to novelty were also submitted by the appellant with its statement of grounds of appeal.

10. These new lines of argument are not to be considered, since the data disclosed in both tables were lastly found irrelevant in the assessment of an anticipating disclosure of documents D16 and D23 for the subject-matter of claim 1 (see points 36, 37 and 38.2 below).

Admission into the appeal proceedings (Article 12(4) RPBA) of supplementary experimental data shown in Tables 1 to 6 of the Annex (document D29) and lines of arguments based thereon in relation to sufficiency of disclosure (Article 100(b) EPC)

11. The experimental data presented in Tables 1 to 6 (see document D29) and the lines of arguments based thereon in relation to sufficiency of disclosure were likewise submitted by the appellant with its statement of grounds of appeal.

12. In its communication in preparation of the oral proceedings, the board observed that it was not evident that the data in Tables 1 to 6, including any of the appellant's arguments based thereon in relation to sufficiency of disclosure, represented developments of the appellant's previous lines of arguments submitted in the first instance proceedings before the opposition division. Thus, these submissions were considered to be new and reasons why they could have not been submitted in the first instance proceedings were not provided by the appellant in its statement of grounds of appeal. Nor have they been provided by the appellant in response to the board's communication, although this issue was explicitly addressed.

13. According to established case law, the primary function of an appeal is to give a judicial decision upon the correctness of a separate earlier decision taken by a department of first instance, while appeal proceedings are not an opportunity to re-run or to continue opposition proceedings (see Case Law of the Boards of Appeal of the EPO, 8th edition 2016 (hereinafter "CLBA"), IV.E.1).

14. In view of the above, in exercising the board's discretion according to Article 12(4) RPBA, Tables 1 to 6 (see document D29), and any new line of argumentation in relation to sufficiency of disclosure, are not to be considered in the proceedings.

Admission of a new line of argumentation (Article 12(4) EPC) with regard to Article 100(c) EPC

15. The appellant further submitted with its statement of grounds of appeal, that claim 1 comprised added subject-matter contravening Article 100(c) EPC.

16. The board observed in its communication according to Article 15(1) RPBA that the appellant's objection under Article 100(c) EPC was directed against a feature in claim 1, which was not objected by both opponents during the first instance proceedings. Moreover, since claim 1 had not been amended in the opposition proceedings, the appellant's objection under added subject-matter was new. Further, the appellant failed to provide reasons why this objection could not have been raised earlier in the proceedings, such as during the opposition proceedings. Nor has the appellant provided such reasons in response to the board's

communication pursuant to Article 15(1) RPBA, which explicitly addressed this issue.

17. Accordingly, this objection is not to be considered in the appeal proceedings.

Main request (claims as granted)

Article 100(c) EPC

18. The opposition division took the view that the subject-matter of claims 1 to 20 did not extend beyond the content of the application as filed (Article 100(c) EPC; see section II above).

19. The appellant has raised only one objection under Article 100(c) EPC against the method of claim 1, which, as set out above, was not admitted by the board into the appeal proceedings.

20. In these circumstances, there is no reason to deviate from the decision under appeal on this issue.

Claim interpretation - claim 1

21. Claim 1 is directed to a method for selecting a growth rate for controlling the partitioning of a recombinant protein between the supernatant and the periplasm in *E. coli* host cell cultures such that the partitioning of the recombinant protein is most suited to the primary recovery of the recombinant protein, wherein expression of the recombinant protein by said cells is under the control of an inducible system. The method comprises several defined process steps.

22. The physical steps in claim 1 require *inter alia* that a growth rate is selected from the range of 0.0005/h to 0.04/h for controlling the partitioning, i.e. the distribution, of a recombinant protein of interest between the supernatant and the periplasm of *E. coli*. Moreover, since the functional feature "*is most suited to the primary recovery of the protein*" is not further defined, claim 1 requires that any growth rate in the specified range affects the relative partitioning of a protein between the periplasm of *E. coli* and the supernatant in any ratio and extent, be it large or small, as determined by comparing the protein's concentration in these two compartments.

23. Furthermore, claim 1 is directed to a "*method for selecting a growth rate that controls the partitioning of a recombinant protein*", in other words, it is directed to the use of a parameter for attaining a technical effect underlying said use, namely for controlling the partitioning of a protein between *E. coli*'s periplasm and the supernatant. Moreover, the method claim does not include physical steps resulting in the production of a product. In these circumstances, the technical effect is interpreted as a functional technical feature that limits the scope of the method (see e.g. T 848/93 of 3 February 1998, point 3.2 of the reasons).

Sufficiency of disclosure (Article 100(b) EPC) - claim 1

24. In light of the board's decision above not to admit into the appeal proceedings any of the new data in Tables 1 to 6 (see document D29) including any of the appellant's lines of arguments submitted under

insufficiency, the assessment of this issue is limited to the facts and arguments dealt with in the decision under appeal.

25. The board agrees with the finding of the opposition division in the decision under appeal that the patent discloses the invention as defined in claim 1 in a manner sufficiently clear and complete for it to be carried out by a skilled person.

26. Examples 1 and 2 of the patent disclose two methods for controlling the growth of *E. coli* cells to obtain growth rates falling within the range recited in claim 1. Example 1 describes for this purpose the use of a limiting glycerol feed (see paragraphs [0036], [0040] and [0041]), Example 2 the use of limiting phosphate concentrations (see paragraphs [0042], [0047], and [0049]). Moreover by determining and comparing the yields of a recombinant Fab protein in *E. coli*'s periplasm and the supernatant according to the instructions disclosed in paragraph [0037] and Figures 2 and 5 of the patent, the skilled person could select growth rates resulting in a desired relative partitioning of the Fab protein between these two compartments.

27. Furthermore, Examples 1 and 2 report that **all** of several growth rates falling within the range of 0.0005/h to 0.04/h as recited in claim 1 affect the relative distribution of a secreted Fab fragment, as an exemplary protein, between *E. coli*'s periplasm and the supernatant (see Tables 1 and 3, Figures 3 and 6).

28. Figures 3 and 6 disclose that at **low** growth rates (i.e. at glycerol feed rates of 0.5 to 2.1 ml/h (corresponding to growth rates of -0008/h to 0.0075/h,

see Table 1), or at phosphate concentrations of 26.9 to 29.8 mM (corresponding to growth rates of 0.0124 to 0.0178/h, see Table 3)), Fab protein is primarily secreted into the **periplasm**. Contrary thereto at **higher** growth rates (i.e. at glycerol feed rates of 5.4 and 10.9 ml/h (corresponding to growth rates of 0.0289 and 0.0374/h, see Table 1), or phosphate concentrations of 31.2 to 35.6 mM (corresponding to growth rates of 0.0191 to 0.0337/h, see Table 3)), relatively more Fab protein compared to total protein is secreted into the **supernatant**. Since the partitioning of the Fab protein between periplasm and supernatant changes at each of the growth rates tested, all of the growth rates falling within the range referred to in claim 1 are suitable for controlling the partitioning of the protein. This enables the skilled person to select a growth rate according to a desired primary recovery of the protein.

29. In the context of inventive step, the appellant submitted that the patent failed to demonstrate that growth rates falling within the range recited in claim 1 have any effect on the partitioning of the recombinant protein between the periplasm and the supernatant.

29.1 The board notes that the contested feature "*selecting a growth rate from the comparison made in step (e) in which the partitioning of the recombinant protein between the supernatant and the periplasm is most suited to the primary recovery of the recombinant protein*" in claim 1 relates to a technical effect. In other words, step f) defines a functional feature of the claimed method. In these circumstances, it is established case law that the question of whether or not the desired effect is achieved by substantially all

features (here growth rates) falling within the scope of the claim, is generally an issue of sufficiency of disclosure (see decision G 1/03, OJ EPO 2004, 413, point 2.5.2 of the Reasons).

29.2 For the reasons set out above and in view of the experimental data disclosed in Tables 1, 3 and Figures 3 and 6 of the patent, the appellant's argument is not convincing.

30. In a further line of argument in the context of inventive step, the appellant submitted that the teaching in the patent invited the skilled person to embark on a research program, since he or she had to test by trial and error firstly various feed rates to obtain growth rates in the range recited in claim 1, which then all had to be assessed for a potential effect on the partitioning of a protein of interest between the periplasm or the supernatant, before a decision could be taken to select a desired growth rate for the protein's primary recovery. However, since the patent was silent about the conditions under which the tests were to be performed, except for a specified range of growth rates, the testing amounted to an undue burden for the skilled person.

30.1 These arguments are not convincing either, since as submitted by the appellant itself, the empirical testing of culture conditions affecting *E. coli*'s growth rate belongs to the skilled person's common general knowledge (see e.g. patent, paragraphs [0020] and [0021]). The same applies for the determination of a protein concentration in the periplasm and the supernatant. Moreover as indicated above, Examples 1 and 2 in the patent disclose that various growth rates falling within the range recited in claim 1 affect the

partitioning of recombinant Fab at least to a small extent (see Figures 3 and 6 in the patent and points 27 and 28 above). Thus, the skilled person, by performing standard tests, can establish growth rates falling within the range recited in claim 1 showing an effect on protein partitioning between the periplasm and the supernatant without undue burden.

30.2 Moreover, the appellant has not submitted evidence that any of the growth rates falling within the range of 0.0005/h to 0.04/h as recited in claim 1 do not affect the partitioning of a recombinant protein between the supernatant and the periplasm of an *E. coli*. In the absence of any verifiable facts, the appellant has not discharged its burden of proof (see CLBA, II.C.8).

31. Thus, the patent discloses the claimed subject-matter in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

Novelty (Article 100 EPC in conjunction with Article 54 EPC) - claim 1

32. The appellant submitted that the method of claim 1 was anticipated by the disclosure of documents D14, D16 and D23.

33. As set out above, claim 1 relates to a method which requires growth rates in the range of "0.0005 to 0.04/h" to be selected that control/affect the relative distribution/partition of the protein between the periplasm and the supernatant at any ratio as determined by the protein's yield in these two compartments.

34. Document D14 discloses the continuous overproduction of a recombinant β-lactamase protein in an inducible system by *E. coli* cultivated in a two stage chemostat fermentation process. The protein is secreted into the supernatant (see abstract). The yield of the β-lactamase protein is assessed in the second stage of the chemostat by comparing "*the β-lactamase-specific activity in the whole culture (total) and in the culture medium (excreted) at each dilution rate*" (emphasis added) selected from "*0.060, 0.045, 0.03, and 0.015 /h*" which is changed compared to the dilution rate in the first stage that is "*fixed at 0.12 /h*" (see page 939, column 2, first full paragraph to page 940, column 1, first paragraph, Figure 2, and Table I). In other words, document D14 discloses the determination of the active β-lactamase's yield in samples of the culture medium, i.e. the supernatant, and the whole culture.

34.1 It is common ground between the parties that the term "*culture medium*" disclosed in document D14 equals the supernatant recited in claim 1. However, it is a matter of dispute whether or not the term "*whole culture (total)*" in the passage above is identical to the term "*periplasm*" recited in step d) of claim 1 or rather relates to a sample which comprises the supernatant and disrupted cells composed of *E. coli*'s periplasm and cytoplasm.

34.2 Document D14 discloses with regard to whole culture samples that the "*β-Lactamase activity (whole culture lysate, defined as "total") was determined after rupturing cells (French pressure cell, Aminco) at 20,000 psi*" (see page 938, column 2, first paragraph, emphasis added).

34.3 The document further reports in the context of protein yield in the whole culture sample that "*Within the range of dilution rates studied, a second stage dilution rate of 0.030 h^{-1}, or 33-hour residence time, most closely approximates the optimum where steady production of ~600 units β-lactamase/mg total protein was observed [Fig. 2(c)]*" (see page 940, column 1, first paragraph). In other words, at a dilution rate of 0.030/h, the concentration of β-lactamase reaches its maximum of about 600 units in the whole culture sample.

34.4 Concerning the level of secretion of β-lactamase into the supernatant, document D14 mentions that "*Excretion of β-lactamase from RB791(pKN) was found to be associated with high rate β-lactamase synthesis.[30] An increased level of excretion was associated with dilution rates which yield higher whole culture activity. At D_2 = 0.030 h^{-1}, the highest level of excretion, 60% to 70%, was observed [Fig. 2(c)]*" (see page 940, column 2, second paragraph, emphasis added). Figure 2(c) discloses in this context that about 400 units of β-lactamase/mg total protein are secreted at a dilution rate of 0.030/h, resulting in a secreted level of "*60% to 70%*" (400 units of secreted β-lactamase represent about 67% of the 600 units of total β-lactamase in the whole sample as set out above). In other words, the document teaches that **under optimal growth conditions the majority of the active enzyme is secreted into the supernatant**.

34.5 Figure 2 discloses that the yield of **total** β-lactamase in **the whole culture**, is higher than that in the **supernatant**, although as set out above, the majority of the enzyme is found in the supernatant. It necessarily follows from this observation that the β-lactamase concentration in the whole culture sample relates to

the sum of enzyme present in the supernatant and the ruptured cells.

34.6 Accordingly, document D14 discloses directly and unambiguously that the **β-lactamase determined in the whole culture** sample **relates to the yield derived from the supernatant and the ruptured cells**. In these circumstances, the question whether or not the yield of active β-lactamase determined in the whole culture sample equals that of the periplasm only, i.e. does not contain any cytoplasmic enzyme from the ruptured cells, is considered irrelevant for the assessment of novelty. The same applies to the issue of whether or not the "dilution rate" disclosed in document D14 is identical to a "growth rate" as recited in claim 1.

34.7 Thus, document D14 does not anticipate the method according to claim 1, because it does not disclose that the protein yield is determined in the periplasm and the supernatant as specified in step d) of claim 1.

35. Document D16 discloses that the production of a recombinant Fab protein in *E. coli* is affected by inducer concentrations and the temperature (see abstract). The document reports in this context that "*Under standard conditions (37°C, 1 mM IPTG), production of functional Fab was very low. At the optimal conditions of 30°C and 0.1 mM IPTG, however, we found that the total amount of functional Fab produced was up to 3.68 mg/l culture during a 3 h incubation. This amount is 12.7 times higher than that obtained under standard conditions*" (see page 354, column 2, fourth paragraph, Table 1, emphasis added). The document further indicates that "*Secretion patterns corresponding to the bacterial growth in the flask are shown in Fig. 2. During the first 8-10 h after*

induction, the secreted Fab was accumulated in the periplasmic space and the medium. During the 10-22 h period, the amount of product in the periplasm gradually decreased, but that in the culture medium continued to increase. The maximum amount of functional Fab, about 4.5 mg/l culture, was observed 10 h after induction" (see page 354, column 2, last paragraph to page 355, column 1, first paragraph, emphasis added).

36. In the board's view, the skilled person would derive from the passages in document D16 set out above that the selection of a particular temperature ("*30°C*") and inducer concentration ("*0.1 mM IPTG*") optimises the production and, hence, the secretion of recombinant Fab into the supernatant. However, these passages are silent on selecting "***a growth rate for controlling the partitioning of a recombinant protein into the supernatant and the medium***" as recited in claim 1. The same applies to Figure 2 of document D16.

37. Since document D16 fails to disclose step f) of claim 1, the issue of whether or not the data in Table 7 of the Annex (see document D29) reflect truly the implicit growth rate values in the growth curve disclosed in Figure 2 of document D16 is irrelevant for the assessment of novelty. Even if they did, document D16 discloses not all of the technical features referred to in claim 1.

38. Document D23 discloses a secretion system for heterologous proteins produced in *E. coli* using a bacterial release protein named "*kil*" under the control of a stationary-phase promoter (see abstract). After induction of the kil gene during the stationary growth phase, recombinant proteins located on the same expression construct are predominantly secreted from

the periplasm into the supernatant due to the action of the kil protein (see also Figure 4 and page 147, column 2, last paragraph).

38.1 The document further reports that the expression of the kil gene *is induced automatically at the beginning of the stationary phase. 3. Therefore, neither manual control of cell density for determining the induction time nor the addition of chemicals for the induction of kil expression is necessary*" (see page 149, third and fourth paragraph, emphasis added). Thus, document D23 suggests the use of the kil protein, for controlling the partitioning of a recombinant protein between *E. coli*'s periplasm and the supernatant, while it is silent on "*selecting a growth rate for controlling the partitioning of a recombinant protein between the supernatant and the periplasm*" as recited in claim 1.

38.2 Since document D23 fails to disclose step f) of claim 1, the issue of whether or not the data in Table 8 of the Annex (document D29) truly reflect growth rate values implicitly disclosed in Figure 4A of document D23 is considered irrelevant for assessing novelty.

39. Thus, the subject-matter of claim 1 is novel (Article 54 EPC).

Inventive step (Article 100 EPC in conjunction with Article 56 EPC) - claim 1

Closest prior art

40. The appellant considered the disclosure of either of documents D12, D2 or D3 as the closest prior art for the assessment of inventive step of the subject-matter

of claim 1, the opposition division that of document D2.

41. The closest prior art is generally a prior art document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most technical features in common, i.e. requiring the minimum of structural modifications (see CLBA, I.D.3.1).

42. Document D12 investigates the production efficiency of two different recombinant proteins in *E. coli* cultured under fed-batch conditions at high cell densities. It reports in this context that "*Via exponential feeding in the two phase fed-batch operation, the specific cell growth rate was successfully controlled at the desired rates and the fed-batch mode employed is considered appropriate for examining the correlation between the specific growth rate and the efficiency of recombinant product formation in the recombinant E. coli strains*" (see abstract, emphasis added).

43. In other words, document D12 examines the effects of growth rates on increasing the production rate of various heterologous proteins in *E. coli* under defined culture conditions. However, the document fails to disclose a selection of growth rates for controlling the partitioning of a recombinant protein between the supernatant and the periplasm, i.e. the purpose underlying the method according to claim 1.

44. Document D2 discloses that growth rates selected from a range of 0.05 to 0.6/h affect the lipid composition of cell membranes of *E. coli* grown under continuous conditions. It mentions that at low growth rates cell membranes are more rigid and, hence, mechanically more

stable compared to cells grown at higher growth rates. In line with the observed less stable cell membranes at higher growth rates, the document reports that the *E. coli* cells leak (secrete) proteins from the periplasm into the supernatant (see abstract, page 387, column 2, fourth paragraph, page 388, column 1, fourth paragraph). In other words, the document discloses that the growth rate affects the partitioning of a recombinant protein between *E. coli*'s periplasm and the supernatant by changing the lipid composition of the bacterial membranes (see page 387, column 1, first paragraph).

45. Document D3 investigates the impact of growth rates on lipid membrane compositions of *E. coli* cells grown under continuous (like in document D2) and fed-batch culture conditions, and as a function thereof the leakage of the periplasmic protein β-lactamase into the supernatant. Document D3 reports that membranes of cells grown under fed-batch conditions remain stiff, irrespective of whether or not the cells are cultivated at high or low growth rates (see abstract). Thus, contrary to *E. coli* cells grown under continuous conditions, periplasmic protein leakage in cells grown under fed-batch conditions remains stable, i.e. not affected by different growth rates (see Figure 7A). The conclusions reached in document D3 based on these observations are that "*optimisation of protein leakage can only be achieved in the continuously cultivated cell where leakage is twice as high compared to the constant leakage level in fed-batch. If leakage is undesired, continuous cultivation is also preferred since it can be designed to lead to the lowest values detected*" (see abstract, emphasis added). In other words, document D3 discloses that growth rates do not significantly affect protein partitioning in *E. coli*

cells grown under fed-batch conditions. The document rather suggests that for the partitioning of proteins between periplasm and supernatant growth rates of cells should be optimised that are cultivated under continuous conditions, i.e. according to the method of document D2.

46. In the light of the above, only the disclosure in document D2 aims at a purpose similar to that underlying the claimed method. Thus, in line with the criteria set up by the case law (see above), the board agrees with the opposition division that document D2 represents the closest prior art.

47. The appellant submitted that the claimed method differed from that disclosed in document D2 in two features. Firstly, in that lower growth rates (i.e. 0.0005 to 0.04/h, see step b)) were selected to control/affect protein partitioning, compared to a range of 0.05/h to 0.6/h as disclosed in document D2 (see page 387, column 2, fourth paragraph). Secondly, while claim 1 requires that the expression of the recombinant protein is induced (see step c)), the recombinant protein disclosed in document D2 is constitutively expressed. It was contested by the appellant that any of the two distinguishing features were associated with a technical effect.

48. As set out above under sufficiency of disclosure, Examples 1 and 2 of the patent disclose that each of the various growth rates tested, all falling within the range of 0.0005 to 0.04/h recited in claim 1, affect the protein's partitioning between the supernatant and the periplasm of *E. coli*. Thus, the board cannot agree with the appellant that the specified growth rates in claim 1 are not associated with a technical effect.

However, there are no data on file demonstrating that a growth rate selected from the range recited in claim 1 affects the partitioning of proteins to a larger extent than a growth rate falling within the range disclosed in document D2, or that an induced gene expression offers advantages over a constitutive gene expression.

49. Thus, in the absence of evidence of such advantageous effects, the technical problem resides in the provision of an alternative method for selecting a growth rate for controlling the partitioning of a recombinant protein between the supernatant and the periplasm in *E. coli*.

50. In view of the experimental data reported in Examples 1 and 2 of the patent and the reasons provided under sufficiency of disclosure above, the subject-matter of claim 1 provides a solution to this technical problem over substantially the whole of the claimed ambit.

Obviousness

51. It remains to be assessed whether or not the skilled person starting from the closest prior art method in document D2 and faced with the technical problem identified above, would have arrived at the claimed method in an obvious manner.

52. Document D2 discloses that a growth rate of 0.3/h in the tested range of 0.05/h to 0.6/h is particularly suitable for secreting recombinant β-lactamase into the supernatant (see Figure 6B). Furthermore, Figures 6A and B show that at low growth rates (0.05/h to 0.015/h), the production of total protein including β-lactamase is low; almost no β-lactamase is detectable in the supernatant; cell lysis is at a maximum level,

(see also page 390, column 2, first paragraph). The abstract of document D2 summarises these results as follows: "*The mechanical strength towards sonication and osmotic shock/enzymatic treatment showed that the cells were more rigid at low dilution rates. However, this was accompanied by a higher cell lysis, a reduced capacity for total and specific protein production and a lower yield of cells*" (emphasis added).

53. This statement in document D2 rather deters the skilled person from using growth rates lower than 0.05/h for controlling the partitioning of recombinant proteins, because the overall protein production rate under these conditions is very low. Furthermore, document D2 provides no hints that growth rates lower than 0.05/h, for example, in the range cited in step b) of claim 1, have an effect on the partitioning of proteins between the periplasm and the supernatant at all. Rather on the contrary, due to the observed more rigid cell membranes at low growth rates, no protein partitioning to the supernatant could be expected (see points 28 and 52 above).

54. The appellant submitted that the skilled person was motivated to use low growth rates because they were preferred for industrial production purposes. The board is not convinced by this argument, since the passage in document D2 relied on by the appellant in support of its case reads: "*The optimum conditions today for high cell density cultivation result in cells, while in the production state, being cultivated at very low growth rates to increase productivity. As indicated above, there are several negative effects for this production state. In summary, these are: increased lysis, highest accumulation of endotoxin, membrane stiffness to mechanical treatment and osmotic permeabilisation and*

low leakage. *These are criteria that all have to be taken into consideration for the optimisation of cultivation and the primary recovery steps of purification.*" (see page 391, column 2, last paragraph, emphasis added).

55. Thus, although the passage reports an increased productivity of recombinant proteins in cells cultivated "*at very low growth rates*", the statement contradicts the experimental data disclosed in Figure 6 of document D2, which show that at low growth rates in the range of 0.05/h and 0.15/h the total protein production rate as well as that of β-lactamase is at **its minimum**. Moreover, the term "*very low growth rates*" has a relative meaning and is not defined by values to which it relates. Since the passage above also mentions disadvantages in relation to the low growth rates, it is not convincing that it provides a pointer for the skilled person to use growth rates even lower than 0.05/h for controlling protein partitioning. Thus, the claimed method cannot be considered obvious for the skilled person based on the teaching of document D2.

56. Document D3 discloses the identical range of growth rate (0.05/h to 0.6/h, see e.g. Figure 7A and B) as indicated in document D2 above. Moreover, in view of the reported failure of growth rates selected from this range to affect the partitioning of β-lactamase into the supernatant at all, document D3 rather deters the skilled person from using growth rates lower than 0.05/h to solve the underlying technical problem.

57. Lastly, as set out above, document D12 relates to a different technical purpose, namely how to improve the production efficiency of recombinant proteins in *E. coli* by controlling its cell growth (see e.g. page 366,

column 1, third paragraph). Therefore, the document provides no pointer to the skilled person to arrive at the claimed method either.

58. Consequently, the subject-matter of claim 1 involves an inventive step (Article 56 EPC).

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated