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Datasheet for the decision of 28 July 2016

Case Number: T 1206/13 - 3.3.08

Application Number: 04752591.0

Publication Number: 1623019

IPC: C12N5/02

Language of the proceedings: EN

Title of invention:
RESTRICTED GLUCOSE FEED FOR ANIMAL CELL CULTURE

Patent Proprietor:
Wyeth LLC

Opponent:
F. Hoffmann-La Roche AG

Headword:
Low levels of lactic acid production in mammalian cell culture/WYETH LLC

Relevant legal provisions:
EPC Art. 123, 83, 54(3), 56, 114(2)
RPBA Art. 12(4)

Keyword:
Main request - requirements of the EPC met (yes)

Decisions cited:

Catchword:

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Case Number: T 1206/13 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 28 July 2016

Appellant I: (Patent Proprietor)	Wyeth LLC 235 East 42nd Street New York, NY 10017-5755 (US)
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Appellant II: (Opponent)	F. Hoffmann-La Roche AG Grenzacherstr. 124 4070 Basel (CH)
Representative:	Jaenichen, Hans-Rainer Vossius & Partner Patentanwälte Rechtsanwälte mbB Siebertstrasse 3 81675 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 18 March 2013 concerning maintenance of the European Patent No. 1623019 in amended form.**

Composition of the Board:

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

Summary of Facts and Submissions

I. Both, the patent proprietor (appellant I) and the opponent (appellant II) filed an appeal against the decision of the opposition division whereby European patent No 1 623 019 with the title "Restricted glucose feed for animal cell culture" was maintained in amended form. The opposition division decided that the main request filed on 5 December 2012 did not meet the requirements of Article 54(3) EPC but that auxiliary request 1 (originally filed as auxiliary request 2 with letter of 5 December 2012) met the requirements of the EPC.

II. With its statement setting out the grounds of appeal appellant I submitted a main request and auxiliary requests 1 to 14. Appellant II, with its statement, submitted new documents D72 to D74.

III. Both parties replied to the other party's statement of grounds of appeal.

IV. The parties were summoned to oral proceedings. A communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed to the summons, informed them of the preliminary non-binding opinion of the board on some of the issues of the appeal proceedings.

V. With a further submission, Appellant II filed additional comments and new document D75. Appellant I filed further comments, withdrew several of the pending claim requests and renumbered the remaining ones. With a final submission dated 22 July 2016, appellant I filed alternative versions of the main request and of auxiliary request 1.

VI. Oral proceedings were held on 28 July 2016. In the course of the proceedings, appellant I made the request labelled "Main Request (amended dependency)" filed on 22 July 2016 its main request.

VII. Claims 1 and 2 of the main request read:

"1. A cell culture method for controlling lactic acid production to low levels in a fed-batch cell culture comprising:

mixing animal cells and a medium to form a cell culture; and
feeding glucose in a restricted manner to the cell culture,

wherein feeding glucose in a restricted manner comprises providing glucose to the cell culture at a rate that is a function of an expected or a premodeled rate of glucose consumption by the animal cells cultured in medium containing a high level of glucose,

wherein the function is multiplication of the expected rate or premodeled rate by a percentage less than 100%, and,

wherein the percentage is no more than 45%, and

wherein a pH sensor is used to monitor pH of the cell culture, and, in response to a rise above a predetermined pH value, additional glucose is fed in a restricted manner to the cell culture.

2. The method of claim 1, wherein the percentage is at least 33%."

Dependent claims 3 to 23 define specific embodiments of the cell culture method of claim 1.

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

D2: WO 2004/048556;

D6: Hu W.S. et al. (1987), Develop. Biol. Standard 66, 279-290;

D7: Lee J. et al. (1999), Biotechnology Advances 17, 29-48;

D16: Cruz H.J. et al. (2000), Journal of Biotechnology, 80, 109-118;

D26: Glacken M.W. et al. (1986), Biotechnology and Bioengineering XXVIII, 1376-1389;

D37: Gambhir A. et al. (1999), Journal of Bioscience and Bioengineering 87, 805-810;

D55: Oh G.-S. et al. (1996), Journal of Fermentation and Bioengineering 81, 329-336;

D74: "Biochemistry", Voet & Voet (1995) 2nd edition; pages 445, 464 and 466;

D75: Declaration of Dr. Thomas Link.

IX. The arguments of appellant I, as far as they are relevant to the present decision can be summarized as follows:

Admission of the main request

The main request differed from the main request previously on file by amended back references in claims 6 and 7. This amendment was supported by claims 9 and 10 as originally filed.

Article 123(2) EPC

The subject matter of claim 2 was directly and unambiguously derivable from paragraph [0015] but also from paragraph [0017] of the application as filed. These paragraphs disclosed the feeding of glucose at a rate which is at least 33%, or no more than 45% of an expected rate. This was true for the "*expected*" and the "*premodeled*" rate. The "*at least 33%*" defined a range from 33% to 100%, the "*no more than 45%*" defined a range from 0 to 45%. The range from 33% to 45% was implicitly disclosed by the combination of the endvalues of these two ranges. This interpretation was in line with the case law, for instance decision T 2/81 of 1 July 1982.

Article 123(3) EPC and Rule 80 EPC

Insertion of the word "*premodeled*" in the term "*wherein the function is multiplication of the expected or premodeled rate*" did not extend the scope of the patent as granted. While claim 1 as granted encompassed a rate of glucose delivery based on any function of a premodeled rate, claim 1 of the main request was limited to methods wherein said function was restricted to multiplication of the premodeled rate by a percentage less than 100%. Claim 1 complied with the requirements of Article 123(3) EPC and Rule 80 EPC.

Article 83 EPC

The process of claim 1 combined two strategies in order to restrict glucose in a fed-batch cell culture. It combined modeling a consumption rate for determining the amount of glucose provided as a pre-programmed base feed (open loop) with a specific closed-loop circuit (monitoring the pH increase). The base feed was obtained by applying the 45% restriction to rates determined or known from a high-glucose culture model (reference rate). The closed loop circuit however did not apply the "*45% model*", and depending on the actual pH measured during culture, additional glucose might, or might not, be provided to the cells. In other words, this aspect was not premodeled, but reactive. The only limitation to the second strategy was that the additional glucose was fed in a restricted manner to the cell culture. It was within the capacity of the skilled person to define the amount of additional glucose to be provided depending on the specific parameters of the cell culture. When determining the amount of additional glucose, the skilled person would have in mind the purpose of the process which was to restrict the quantity of glucose provided to the cell. Therefore, it would select the amount of additional glucose with the aim of preventing glucose starvation while at the same time maintaining the glucose restriction to keep lactic acid at low levels. Further guidance could also be found in paragraphs [0053, 0059 and 0060] of the patent with the effect that the method was fully operable.

Admission of documents D72 to D75

Document D75 could be admitted into the proceedings, documents D72 and D73 should not be admitted. Document

D72 was no more relevant than document D2. It should and could have been submitted earlier in the opposition proceedings in view of its publication date and the close association of its author with the opponent. Document D74, an illustration of common knowledge of the skilled person, was not highly relevant for the case at issue and should not be admitted.

Article 54(3) EPC

The claimed method required the use of a pH sensor to provide glucose if needed. This implied a particular set-up. The mere presence of a pH sensor in the set-up of document D2 did not imply the method step of claim 1.

Article 56 EPC

Document D6 disclosed the programmed continuous feeding of limiting amounts of glucose to reduce lactate levels in the culture medium. Starting from this document as the closest prior art, the technical problem was the provision of an improved method for controlling lactic acid to low levels in cell culture while protecting cells against glucose starvation. This problem was solved by the method of claim 1 as demonstrated by Example 3, and Figures 8 and 9 of the patent.

Only with hindsight, would the skilled person starting from document D6 turn to document D7.

In mammalian cell cultures, lactic acid concentration not ammonium concentration was the dominant factor leading to a change in pH. Therefore, the skilled person would not have been motivated to take the procedure called "*pH-stat*" into consideration which was

described in a single paragraph on page 31 of document D7.

Document D7 had also no particular focus on this procedure. Moreover, the first document referred to in the paragraph on page 31 disclosed a cell culture method of E. coli. It was unclear if the second document mentioned in this paragraph belonged to the field of the cultivation of animal cells. Rather, the whole paragraph referred to the cultivation of bacteria. There was also no mention of lactate or glucose in this paragraph. Finally, most of document D7 referred to alternative more complex control systems which had nothing to do with the use of a pH sensor for the control of glucose feed rates.

The skilled person would rather have turned to a document like D55 which addressed the problem of maintaining lactate and ammonia levels at a low level in mammalian cell cultures. The method described in document D55 involved the use of a pH sensor but only to keep pH constant and not to trigger the feeding of glucose if the pH rose above a certain level. Further documents like D16 and D37 addressed the same problem but none of them comprised the use of a pH sensor to trigger glucose feeding.

X. The arguments of appellant II, as far as relevant to the present decision can be summarized as follows:

Article 123(2) EPC

Claim 2, by reference to claim 1, created a range from 33% to 45% which lacked basis in the application as filed. In paragraphs [0015, 0017, and 0050] of the patent application, the two values "*33%*" and "*45%*" were

only disclosed as isolated alternative values but not as endpoints of a range. In addition, insertion of the word "*premodeled*" in the term "*wherein the function is multiplication of the expected or a premodeled rate*" created a "*function*" which was 33% to 45% of a premodeled rate. This represented an intermediate generalisation which was not directly and unambiguously derivable from the application as filed. A premodeled rate in combination with a function which was at least 33% and no more than 45% of a determined or premodeled rate was only disclosed in paragraph [0017] of the application as filed. The method disclosed in this and the subsequent paragraph involved however additional steps, i.e. the determination of the glucose consumption rates of animal cells in a culture medium comprising a high level of glucose, which were not included in claim 1. Paragraph [0015] could not serve as a basis because it only related to functions of an "*expected*" but not of a "*premodeled*" rate and the two terms had different meanings.

Article 123(3) EPC and Rule 80 EPC

The insertion of the word "*premodeled*" in the term "*wherein the function is multiplication of the expected or a premodeled rate*" also broadened the scope of claim 1 because it introduced a further option for the possible functions. Moreover, this amendment was not occasioned by a ground of opposition.

Article 83 EPC

The claimed method encompassed areas where the last step could not be performed and the method not be realised. Figure 9 of the patent showed that, with a feed rate of 45%, the glucose concentration fell to low

levels, the lactic acid concentration was however rising continuously. With increasing lactic acid concentrations, the pH was falling continuously and no rise of pH about a predetermined level could ever be detected. This meant that at a feeding rate of 45% no additional glucose could be fed and lactic acid levels could not be controlled to low levels according to the preamble of the claim. At a feed rate of 33%, lactic acid levels fell a little bit which might trigger the addition of a glucose bolus. This change in pH depended on the buffer system used. The patent gave however no guidance concerning the buffer system. If the method was performed with 0% glucose feed rate, no lactic acid would be produced, the pH would not change and the last step of the method would not be performed at all. Moreover, the claimed method was not illustrated by any example. In the examples (paragraphs [0080, 0098]) a pH sensor was only used to maintain pH by the addition of a titrant (carbonate/bicarbonate buffer) but not via the addition of glucose.

Admission of documents D72 to D75

Document D72 was prima facie highly relevant for the assessment of novelty. Its content went beyond the content of document D2 and disclosed the use of a pH sensor. At the time of the oral proceedings before the opposition division, the date of its public availability could not be established unambiguously. Therefore appellant II decided not to introduce it into the opposition proceedings. Evidence for the date of its availability was now on file through document D73. Document D74 was filed to illustrate the common knowledge of the skilled person. Document D75 was a declaration of the technical expert, explaining what technical data a skilled person would extract from the

data presented in document D6. It did not introduce anything going beyond the content of document D6.

Article 54(3) EPC

According to page 10, lines 14 to 17 of document D2, the pH of the culture medium was preferably within specified ranges. It was clear from this statement that a pH sensor was used in the methods disclosed in this document since pH sensors were routinely used to control the pH of culture media. If the claimed method was performed with a glucose feed rate of 45%, no additional glucose would be fed, and the method of claim 1 could not be distinguished from the method disclosed in document D2.

Article 56 EPC

Document D6, representing the closest prior art, disclosed all the technical details of the claimed method except for the use of the pH sensor to control glucose addition. Figure 9 showed that the restricted feeding of glucose resulted in reduced lactate concentrations in the culture medium. Figure 8 showed that cell growth was not affected. As explained in document D75, glucose feed rates in document D6 were limited to 11% and 15%, respectively, of the maximally possible consumption rates. Document D6 noted that a possible effect of controlling the glucose concentration at low levels was increased glutamine oxidation which led to the accumulation of ammonium in the culture medium. High ammonium concentrations had an inhibitory effect on cell growth. As shown by document D26, this was a generally known phenomenon. Starting from document D6, the technical problem was the provision of an improved method for controlling lactic

acid to low levels in cell cultures while preventing cells from suffering glucose starvation. The solution to this problem was provided by Document D7 which described advances in the control of fed-batch fermentations. Under the header "*Feed control strategies*", in section 1.1 and Figure 1, a strategy was described where the nutrient feed rate was manipulated to maintain the pH at a set point. This strategy was based on the fact that the pH rises due to the excretion of ammonium ions when the principle carbon substrate is depleted. The pH was routinely measured. The claimed solution was obvious since document D6 addressed the problem of rising ammonium concentrations when lactate concentrations drop and section 1.1. of document D7 addressed exactly this point. The fact that section 1.1. of document D7 possibly related to the cultivation of bacteria did not prevent the skilled person form considering the proposed solution because the cultivation of bacteria belonged to a neighbouring technical field. Whether the rise in pH resulted from the consumption of lactate or the release of ammonium ions made also no difference to the skilled person's approach to solve the technical problem. Both phenomena were generally known. Alternative solutions such as those disclosed in documents D16, or D37 were not as effective as the one proposed in document D7.

Figure 9 of the patent showed that the lactic acid concentration increased constantly at a feed rate of 45% of the maximal possible glucose consumption rate, indicating that no metabolic shift to lactate consumption occurred. The method of claim 1 performed with a 45% glucose feed rate could therefore not achieve the claimed effect of controlling lactic acid production to low levels. Moreover, according to the

priority document, low levels of lactic acid concentrations referred to concentrations below 5 g/l. The term had a particular meaning which, according to decisions T 409/91 of 18 March 1993, T 500/01 of 12 November 2003 and T 620/08 of 4 May 2011, had to be considered when interpreting claim 1. Figure 9 showed however that the level of 5 g/l was exceeded if cultivation was continued beyond 187 hours.

XI. Appellant I requested that the decision under appeal be set aside and the patent be maintained on the basis of the Main request filed at the oral proceedings before the board.

XII. Appellant II requested that the decision under appeal be set aside, the patent be revoked and documents D72 to D75 be admitted into the proceedings.

Reasons for the Decision

Admission of the main request filed at the oral proceedings

1. The main request differs from the main request previously on file (which was identical to auxiliary request 1 underlying the decision under appeal) by amended back references in dependent claims 6 and 7.

2. Claims 6 and 7 of the main request are based on claims 9 and 10 of the application as originally filed. Said claims referred to claim 8 of the application as filed, the features of which have been inserted into claim 1. Therefore, amended claims 6 and 7 refer now to claim 1.

3. Appellant II had no objections to the admission of the main request, and the board decides to admit it into the proceedings.

Article 123(2) EPC

4. The issue to be examined is whether the subject matter of claim 2 is directly and unambiguously disclosed in the patent application as filed in general and in particular in combination with the feature "*function of a premodeled rate*".

5. The international patent application WO2004/104186 (the application as filed) explicitly disclosed a culture method for controlling lactic acid production comprising feeding glucose in a restricted manner. It discloses three alternative functions of feeding the glucose, which encompass glucose concentrations from 0 % to less than 100%, from 33% to less than 100% and from 0 % to 45% glucose (cf. claims 2, 3 and 4, and paragraphs [0015] to [0017]). These three ranges come from a single list. The values of 33% and 45% explicitly define the limits of two subranges and as such also directly and unambiguously disclose the limits of the subrange from 33% to 45% (cf. point 3 of decision T 2/81 of 1 July 1982).

6. It is true that paragraph [0015] explicitly mentions the recited ranges as functions of "*an expected rate*" only.

7. Paragraph [0015] discloses in general terms culture methods for controlling lactic acid production to low levels comprising "*feeding glucose in a restricted manner*". The latter term is further defined in paragraph [0035] of the patent application as providing glucose in an amount that is determined or calculated by a function which is less than 100% of the amount of glucose "*expected or determined*". Further, the function

may be "*a function of an expected rate*" (line 10 of paragraph [0035]) where, according to paragraph [0036], the "*function of an expected rate of glucose consumption*" may include a number of mathematical relationships between an "*expected or premodeled rate of glucose consumption*". This includes relationships wherein the glucose feed rate is the product of "*(1) an expected or premodeled rate of glucose consumption at a point in time*" ... and "*(2) a percentage value less than 100%*".

8. The explicit mention in paragraph[0015] of the term "*feeding glucose in a restricted manner*" and of the three specific ranges of glucose feeding rates in combination with the definition of the term according to paragraphs [0035] and [0036] provides a direct and unambiguous disclosure of the subject matter of claim 2.

9. The main request meets the requirements of Article 123(2) EPC.

Article 123(3) and Rule 80 EPC

10. Claim 1 as granted comprised the feature "*wherein feeding glucose in a restricted manner comprises providing glucose to the cell culture at a rate that is a function of an expected or a premodeled rate*". Claim 1 of the main request further specifies that in the case of a "*premodeled rate*" the function is a multiplication by a percentage less than 100%. This amendment does not contravene the requirements of Article 123(3) EPC, as it limits the possible "*functions*" of a "*premodeled rate*" and does not lead to an extension of the protection conferred by the patent.

11. This limitation was introduced into the claim in order to address novelty objections raised against claim 1 as granted and therefore complies with the provisions of Rule 80 EPC.

Claim interpretation

12. Regarding the function defining the feeding of glucose in a restricted manner, appellant I was of the opinion that the limitation "*wherein the percentage is no more than 45%*" did not apply to the restricted feeding of "*additional glucose*" according to the last step of claim 1 which requires that *"in response to a rise above a predetermined pH value, additional glucose is fed in a restricted manner to the cell culture.*"

13. The board does not agree with this interpretation because the claim provides an explicit definition of what is meant by feeding glucose in a restricted manner. According to this definition, "*feeding glucose in a restricted manner*" means feeding glucose at a rate which corresponds to no more than 45% of the maximal possible uptake rate of the culture.

The "*additional glucose"* which is fed in response to a rise in pH above a predetermined level is also explicitly "*fed in a restricted manner".* It makes no sense to interpret the term "*feeding glucose in a restricted manner*" which occurs twice in claim 1 in two different ways although it is explicitly defined in the claim. The board therefore concludes that the feeding of additional glucose is also limited such that the value of 45% of an expected or premodeled rate is not exceeded.

Article 83 EPC

14. Appellant II argued that the patent provided no example to illustrate the method of claim 1 and that the method was partly inoperable if the initial glucose feed rate was already close to or at 45% of the expected or premodeled rate of glucose consumption.

15. The method of claim 1 comprises the steps of (i) mixing animal cells and a medium to form a cell culture, (ii) feeding glucose in a restricted manner to the cell culture, and (iii) feeding additional glucose in a restricted manner if a pH sensor detects a rise in pH above a predetermined value.

16. Mammalian cell culture systems (step (i)) are known in the art, and it has not been contested that the skilled person knows how to feed glucose to such cultures in a restricted manner (step (ii)) (see e.g. documents D2, D6, D7).

17. The purpose of monitoring the pH of the culture medium is to prevent starvation of the cultivated cells if glucose in the culture medium has been consumed and lactate is consumed as an alternative carbon source (paragraph [0052] of the patent). Paragraphs [0053], [0059] and [0060] of the patent give examples of the delivery of additional glucose if a rise in pH of 0.02 units is detected.

18. Thus, the patent provides exemplary guidance of how to perform the method of claim 1 including step (iii).

19. The board is not convinced by appellant II's argument that the method becomes inoperable if the original glucose feed rate is already close to or at 45%. The only consequence of limiting the initial glucose feed

rate to no more than 45% of an expected rate is that no "*additional glucose*" can be fed if the feed rate is already at this level. This does however not render the method of claim 1 inoperable in the sense that the skilled person could not readily perform it.

20. Moreover, the evidence on file does not support appellant II's argument.

Figure 9 of the patent shows the concentrations of glucose and lactate in cultures of CHO cells producing BMP-2. The concentration of lactate increases continuously when glucose is fed at 45% of the expected rate ("*high ramp (L)*") while it reaches a maximum at about 100 hours and then decreases slightly if glucose is fed at a rate of 33% ("*low ramp (L)*"). In both cases the lactate concentration is considerably lower than the lactate concentration in a control culture ("*control (L)*"). Figure 7 discloses that cell concentration and cell viability of the control culture decrease after about 100 hours while both remain high in the "high ramp" and the "low ramp" experiments.

21. The fact that the lactate concentration in the "high ramp (L)" experiment is rising continuously does however not support the argument that it was rising infinitely because Figure 9 contains no data points after about 187 hours. The argument that it was therefore not possible to "*control the lactic acid concentration to low levels*" as required by the preamble of claim 1 remains speculative and is not based on the available facts.

22. There is also no evidence on file that it would be necessary to feed additional glucose if the initial feed rate is set at 45%. In the "high ramp (L)"

experiment of Figure 9, the lactate concentration increases steadily and a decrease but no rise in pH is detected (see also Table 16 "Titrant usage", and paragraph [0080]). As long as there is no increase in pH, there is no need for the feeding of additional glucose in a restricted manner.

23. If the initial feed rate is set at lower values, e.g. at 33% as shown in Figure 9, additional glucose can be fed at a rate up to 45% of the expected rate.

24. Appellant II's argument that the method could not be preformed if the glucose feed rate was set at 0% is also not convincing because step (ii) of the claimed method requires the feeding of glucose in a restricted manner. This step does not include the feeding of no glucose at all.

25. For these reasons, the invention according to the main request is disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. The requirements of Article 83 EPC are met.

Admission of documents D72 to D75

26. Document D74 is a copy of several pages of a general textbook. According to the established practice of the Boards of Appeal, this document is admitted into the procedure.

27. Document D75 presents calculations by appellant II's technical expert, Mr Link, based on data presented in document D6. Appellant I had no objections to the admission of this document and the board admits it into the proceedings.

28. Document D72 is the PhD thesis of Mr Link, who is also mentioned as an inventor on document D2. The PhD thesis was published in 2004, i.e. several years before oral proceedings before the opposition division were held. Mr Link was present at these oral proceedings where the opponent for the first time requested that document D72 be admitted into the procedure (cf. point 4.2 of the minutes). At the end of the discussion of inventive step it stated however, in response to the explicit question by the chairman of the opposition division as to whether the opponent considered document D72 to be more relevant than document D2, that it no longer wished to introduce document D72 into the procedure (cf. point 6.7 of the minutes).

29. Under these circumstances and in view of the fact that the opponent's action prevented the opposition division from taking a closer look at document D72, the board, exercising its discretion under Article 114(2) EPC in conjunction with Article 12(4) RPBA, decides not to admit document D72 into the appeal procedure. The same applies to document D73 which confirms the date of public availability of document D72.

Article 87 EPC

30. In view of the board's decision concerning the admissibility of document D72, appellant II stated, and the board agrees, that there is no need to examine whether the subject matter of the main request is entitled to the claimed priority date (Article 87 EPC).

Article 54(3) EPC

31. Document D2, filing date 7 November 2003, has a publication date of 10 June 2004 which is after the

filing date of 17 May 2004 of the opposed patent. Based on document D2, a European patent application has been filed and a patent has been granted in 2010. Document D2 is thus prior art according to Article 54(3) EPC.

32. Document D2 discloses a method for the cultivation of mammalian cells for the production of substances characterized by feeding glucose in a limited manner, whereby the degree of glucose limitation defined as the ratio of the actual glucose consumption rate and the maximally known glucose consumption rate is ≤ 0.5 (50%) or ≤0.4 (40%) or ≤0.3 (30%) (claims 1 and 2), particularly preferred ≤0.35 (35%) (page 5, line 33). Data were obtained in a continuous perfusion culture but it is described that the method can also be performed in a fed-batch culture (cf. page 15, lines 16-27).

33. Appellant II argued that, if the method of claim 1 was operated with a glucose feed rate close to or at 45% of the expected or premodeled rate, no additional glucose could be fed due to the limitation in claim 1 and the method could not be distinguished from the method of document D2.

34. There is no explicit disclosure of the use of a pH sensor in document D2. There is a reference to preferred pH ranges of the culture medium (page 10, lines 14 to 16) which in appellant II's view, in an implicit way, discloses the use of a pH sensor to keep the pH in the defined ranges.

35. It may be true that pH sensors are commonly used to control the pH of mammalian cell cultures, but this control usually involves the addition of sodium carbonate/bicarbonate (e.g. paragraph [0080] of the

opposed patent], NaOH (e.g. document D55) or CO_2 (e.g. document D16). Thus, document D2 discloses at best implicitly the use of a pH sensor to maintain the pH in the culture medium at a set level.

36. However, document D2 does not implicitly, or otherwise, disclose the use of a pH sensor to monitor pH and to feed additional glucose in response to a rise of pH above a predetermined value as required by step (iii) of the method of claim 1.

37. Furthermore, document D2 does not directly and unambiguously disclose the feeding of glucose at a rate of 45%. There is only direct and unambiguous disclosure of restricted feeding rates of ≤50%, ≤40%, ≤30%,and particularly preferred ≤35% of the expected maximal consumption rate (cf. page 5, lines 30 to 33; page 15, lines 25 to 27; claims 1, 2 and 4).

38. Since document D2 neither discloses the use of a pH sensor to control the glucose feeding rate nor a feeding rate of 45%, appellant II's novelty objection is without merit.

39. Since no other objections concerning the novelty of the claimed subject-matter have been put forward, the main request meets the requirements of Article 54 EPC.

Article 56 EPC

40. Document D6, representing the closest prior art, discloses the programmed feeding of glucose to mammalian cell cultures which results in a higher cell yield based on the amount of glucose consumed and a lower lactate production ("*Introduction*", second but last sentence). The authors of this document found that

cells grown in medium comprising a reduced glucose concentration needed step feeding of additional glucose to prevent cell death (page 281, second paragraph). In the cell culture receiving step-feeding of glucose, the growth kinetics were very similar to that of a control culture (paragraph bridging pages 281 and 283). Figure 8 discloses the results of an experiment in which cells were fed either limiting or non-limiting amounts of glucose and it was found that both cultures grew at the same rate until the maximum cell concentration was reached. The fraction of glucose converted to lactate was reduced in the culture receiving limiting amounts of glucose (Figure 9). In the last paragraph of document D6, the authors note that: "*By controlling glucose concentration at a low level, the accumulation rate of lactate can be reduced. However, under such conditions the production rate of ammonium is likely to be increased. To avoid the inhibitory effect of metabolites it is desirable to identify the optimum cultivation conditions throughout the cultivation period.*"

41. Starting from document D6, the technical problem to be solved is the provision of an improved method for controlling lactic acid to low levels in mammalian cell culture while protecting cells against glucose starvation.

42. Paragraphs [0015, 0017, 0019, 0052, 0053] of the patent give literal instructions how to perform the method of claim 1, and paragraphs [0059 and 0060] convey that such experiments were performed. However, the patent does not provide experimental data obtained by a method according to claim 1.

43. Appellant II did not dispute that the claimed method solves the underlying technical problem in general and the board considers that this is indeed the case.

44. Appellant II argued however that the technical problem was not solved over the entire scope of claim 1. The claimed effect was not achieved if the method was performed with a glucose feed rate of 45%.

45. Figure 9 of the patent shows that the control of lactic acid to low levels can also be achieved with a restricted feed rate of 45%. The lactic acid levels rise to between 4 and 5 g/l which is considerably lower than the roughly 8 g/l of the control culture, and cell viability and density are largely unaffected with a feed rate of 45% (cf. Figure 7). The cells are therefore neither in danger of reduced growth rates due to the accumulation of toxic substances nor in danger of starvation and the claimed method solves the technical problem when performed with a feed rate of 45%.

46. Appellant II argued furthermore that the claimed solution was obvious because the skilled person, knowing from document D6 that the accumulation of lactate was reduced by controlling glucose at a low level and that the production rate of the inhibitory metabolite ammonium was likely to be increased (last paragraph of document D6), would have turned to document D7 which addressed the problem of ammonium accumulation. In the chapter "*Simple indirect feedback (single-loop) methods*" (page 31) control schemes are described where the nutrient feed rate is manipulated to maintain the pH at a set point. It is explicitly stated that "*The pH-stat with high limit is based on the fact that pH rises due to excretion of ammonium*

ions when the principle carbon substrate is depleted."; and "*When pH is higher than its set point, the on/off controller feeds nutrient to the fermentor at a predetermined rate*".

47. The board considers appellant II's argument that the skilled person would have combined the teaching in document D7 with the closest prior art to be based on hindsight. It is correct that document D7 reviews ways of controlling nutrient supply in fed-batch cultures to prevent overfeeding or underfeeding (page 29, first paragraph, and page 46, "Concluding remarks"), and that the skilled person could have consulted this document for solutions to the technical problem.

48. However, the section cited by appellant II consists of a single paragraph while the vast majority of the document is devoted to alternative, more complex control schemes (cf. the sections entitled "*Nutrient feeding according to glucose uptake or demand*" (page 32), "*Nutrient feeding according to inferred substrate concentration or specific growth rat*e" (page 34), "*Other methods*" (page 35), and "*Manipulation of feed rate using fuzzy control and neural networks*" (page 36)).

49. The skilled person would not have turned to the section cited by appellant II because it did not mention the use of the "*pH-stat*" procedure for the control of the lactic acid production in mammalian cell cultures growing on glucose. There is no hint that the documents cited in this section describe the control of mammalian cell cultures. At least two of the three documents cited in this section describe the culture of E. coli (see the references to the citations "[2]" and "[5]"). The skilled person trying to improve the cultivation of

mammalian cells would therefore rather have turned to the section entitled "*Nutrient feeding according to glucose uptake or demand*" which describes (by reference to a document "[16]", Zhou et al.) the determination of the nutrient feed rate in the cultivation of a mammalian hybridoma cell line by calculating the stoichiometric relation between glucose and oxygen consumption, i.e. on the basis of measurements of the glucose concentration and the oxygen concentration. Document D7 reports that this procedure reduces the accumulation of lactate and extends the growth phase (page 33, bottom).

50. Since the skilled person would not have turned to the section cited by appellant II for the aforementioned reasons, there is no need for the board to further examine appellant II's argument that the skilled person's motivation for considering this section was unaffected by the question whether a rise in pH resulted from a decreasing lactic acid concentration or from an increasing ammonium concentration.

51. As alternatives to document D7, the skilled person could also have directed its attention to other documents cited in this procedure which disclose additional ways to optimize mammalian cell growth and product titre while reducing the levels of the toxic metabolites lactate and ammonium.

52. Document D10 discloses a model-based control strategy for the fed-batch cultivation of mammalian cells. The aim of the control strategy was to maintain low levels of the two main substrates glucose and glutamine during fed-batch mode but not to limit cell growth by too low substrate concentrations. The time span and the efficiency of cell growth could be prolonged as a

result of a reduced production of lactate and ammonium (page 1096, right column, third full paragraph). The control strategy involves off-line measurements of cell-, glucose- and lactate concentrations (page 1100, right column, second full paragraph) followed by calculations of the new feed rates.

53. Document D16 discloses that lactate and ammonium production could be reduced by culturing mammalian cells on limiting amounts of glucose and glutamine. To determine the necessary amounts, nutrient consumption rates were estimated by off-line determination of cell, glucose and glutamine concentration (Page 111, right column, last paragraph). The authors conclude by stating that, when working at very low nutrient concentrations, care must be taken to avoid nutrient limitation which can be achieved by the method disclosed (page 116, final paragraph).

54. Document D26, entitled *"Reduction of Waste Product Excretion via Nutrient Control: Possible Strategies for Maximising Product and Cell Yields in Serum in Cultures of Mammalian Cells"*, recommends to control glucose and glutamine concentrations to reduce ammonium and lactic acid accumulation in the medium (page 1388, right column, last paragraph). In order to control glutamine concentrations, on-line measurements of glutamine and cell concentrations are needed. Cell concentrations are determined by calculating ATP production rates which involves measurements of the oxygen uptake rates and lactic acid productivity with an oxygen probe and a pH sensor to estimate lactic acid production from the amount of base required to maintain a constant pH (page 1383, right column, 2nd full paragraph). On-line glutamine estimation can be achieved by either using an

on-line HPLC or by utilizing a glutamine probe (page 1388, right column, first full paragraph).

55. Document D55 discloses a further method to control the production of lactic acid and ammonia to low levels in fed-batch mammalian cell cultures (page 333, right column). The method involves the restricted feeding of glucose and glutamine. Glucose uptake rates were calculated from the amount of NaOH addition required to keep the pH constant, glucose uptake rates were then used to calculate the glutamine uptake rates. The authors were aware of the problem of glucose starvation and kept the glucose and glutamine concentrations relatively constant

56. Thus, at least documents D10, D16, D26 and D55 disclose solutions to the problem described in the final paragraph of document D6. These solutions and also the one described on page 33 of document D7 all relate to the same technical area, i.e. the field of mammalian cell culture, and are more closely related to the technical problem underlying the invention, than the general statement about the use of a pH sensor on page 31 of document D7. In the absence of any pointer in document D6 to document D7, the skilled person had no motivation to turn to page 31 of document D7 which neither mentions mammalian cells nor glucose. It could and would only do so with knowledge of the method disclosed in claim 1, i.e. with hindsight.

57. In view of the above, the board decides that the subject matter of independent claim 1 and of dependent claims 2 to 23 of the main request meets the requirements of Article 56 EPC.

58. In opposition proceedings, the opposition division decided to maintain the patent on the basis of auxiliary request 1 and a description adapted thereto. Since the main request differs from auxiliary request 1 underlying the decision under appeal only by amended back references in claims 6 and 7, no further adaptation of the description is needed.

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 1 to 23 of the main request received during the oral proceedings of 28 July 2016,

   the description as adapted at the oral proceedings in opposition on 5 February 2013, and

   Figures 1 to 9 of the patent as granted.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated