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Datasheet for the decision of 3 June 2022

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Language of the proceedings: EN

Title of invention:
CELL CULTURE METHOD USING AMINO ACID-ENRICHED MEDIUM

Patent Proprietor:
Chugai Seiyaku Kabushiki Kaisha

Opponents:
Thomae, Robert Florian
Neuefeind, Regina
Hoffmann Eitle
Storz, Dr. Ulrich
Oetke, Cornelia

Headword:
A method of culturing a cell/CHUGAI SEIYAKU KABUSHIKI KAISHA

Relevant legal provisions:
EPC Art. 123(2)
RPBA Art. 12(4)

Keyword:
Main request and auxiliary requests 1 to 3 - Article 123(2) EPC (no)
Selection from two lists, see points 2.14 to 2.17 of the reasons.

Decisions cited:
T 0012/81, T 0947/05, T 0860/00, T 0686/99

Catchword:

Europäisches Patentamt
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Beschwerdekammern

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Case Number: T 2836/19 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 3 June 2022

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Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 5 August 2019 revoking European patent No. 2154244 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	A. Bacchin

Summary of Facts and Submissions

I. European patent No. 2 154 244 is based on European patent application No. 08 752 120.9, (published as WO 2008/136398 on the 13 November 2008). The patent was opposed on the grounds of Article 100(a) in conjunction with Articles 54 and 56 EPC, and of Articles 100(b) and (c) EPC. An opposition division decided that the main request and auxiliary requests 1 to 10 infringed Article 123(2) EPC, that auxiliary requests 11 and 12 lacked clarity and that auxiliary requests 13 and 14 did not fulfil the requirements of Article 56 EPC. The patent was revoked.

II. The patent proprietor (appellant) lodged an appeal. With its statement of grounds of appeal, it submitted a main request and auxiliary requests 1 to 3. The main request and auxiliary request 1 are identical to auxiliary request 13 and 14 respectively, submitted during the opposition proceedings. Auxiliary requests 2 and 3 are new.

III. The respondents I to V (Opponents 1 to 5) replied to appellant's statement of grounds of appeal.

IV. In reply to the respondent's responses, appellant filed further submissions.

V. The parties were summoned to oral proceedings. In a communication issued in preparation of the oral proceedings, the parties were informed of the board's provisional, non-binding opinion on some of the legal and substantive matters of the case.

VI. With a letter dated 3 May 2022 appellant replied to the board's communication. With a letter dated 18 May 2022, appellant responded to Opponent 2's submission dated 5 May 2022.

VII. Oral proceedings took place on 3 June 2022.

VIII. Claim 1 according to the main request reads as follows:

"1. A method of culturing a CHO cell by fed-batch culture, comprising adding a fed-batch medium comprising serine, cysteine and tyrosine, wherein the fed-batch medium is fed into the culture solution in multiple batches sequentially or continuously, characterized in that the concentration of serine in the culture solution is maintained at 2 mM or higher by addition of the fed-batch medium, and the concentration of tyrosine in the culture solution is maintained at 1 mM or higher by addition of the fed-batch medium, wherein the concentration of serine and tyrosine are maintained during a period from a fourth day to a tenth day of the culture and wherein the concentration of cysteine in the culture solution is maintained at 0.4 mM or higher by addition of the fed-batch medium, wherein said concentration of cysteine is maintained during a period from a fourth day to a tenth day of the culture, wherein said method is used in a process comprising culturing a cell capable of producing a desired protein to obtain the desired protein."

Claims 2 to 4 are directed to preferred embodiments of claim 1. Claim 5 is directed to a preparation process of a medicament comprising a protein as an active ingredient comprising the step of producing the protein by the process of any one of claims 2 to 4.

Auxiliary request 1 (claims 1-5)

Auxiliary request 1 is identical to auxiliary request 14 of the decision under appeal. It differs from the main request in that claim 1 was amended to include "... wherein the fed-batch medium comprises 50-200 mM L-serine, 10-100 mM L-tyrosine and 1-10 mM L-cysteine hydrochloride monohydrate and is fed into the culture solution ..."(emphasis added).

Auxiliary request 2 (claims 1-2)

Auxiliary request 2 differs from the main request in that claim 1 was amended to combine the features of dependent claims 2 to 4: "A process of producing a desired protein by culturing a CHO cell, comprising culturing a CHO cell using a method ... wherein the cell is transformed with a gene encoding the desired protein, wherein the desired protein is an antibody." (emphasis added).

Auxiliary request 3 (claims 1-2)

Auxiliary request 3 differs from Auxiliary request 1 in that claim 1 was amended to combine the features of dependent claims 2 to 4:
"A process of producing a desired protein by culturing a CHO cell, comprising culturing a CHO cell using a method ... wherein the fed-batch medium comprises 50-200 mM L-serine, 10-100 mM L-tyrosine and 1-10 mM L-cysteine hydrochloride monohydrate and is fed into the culture solution ... wherein the cell is transformed with a gene encoding the desired protein, wherein the desired protein is an antibody." (emphasis added).

IX. The substantive submissions made by the **appellant**, insofar as relevant to the present decision, may be summarized as follows:

Main request (claims 1-5)
Article 123(2) EPC

Appellant agreed with points 2 and 7 of the decision under appeal in view of Article 123(2) EPC.

The method according to claim 1 and the following features:

(a) "adding a fed-batch medium comprising serine, cysteine and tyrosine",
(b) "wherein the concentration of cysteine in the culture solution is maintained at 0.4 mM or higher" and
(c) "maintaining the concentration of serine, tyrosine and cysteine during a period from a fourth day to a tenth day of the culture by addition of the fed-batch medium"

had a basis at least in claims 1 to 16, 19 to 20, 25, 21 to 24 and paragraphs [0010], [0022], [0024], [0028], [0031], [0036], [0038] and [0042] of the patent application.

The addition of a fed-batch medium was disclosed in claims 15, 16 and paragraph [0036], while the concentration of serine, tyrosine and cysteine was explicitly maintained during a period from a third day to a tenth day of the culture. The expression "at least a part of or an entire culture period from the third day to the tenth day of the culture" referred to a culture period from the fourth, fifth, sixth, or

seventh day of the culture, and the selection of a period which extends from the fourth day of culture (see paragraphs [0022], [0026], [0028] of the patent application) amounted to a selection from one list. The working experiments 1 to 3 confirmed that the concentration of serine and tyrosine and/or cysteine in the culture solution were adjusted and maintained by addition of a fed-batch medium (see paragraphs [0015], [0017] and [0016]). This concept was further elaborated in that the concentrations of these amino acids were maintained from a fourth day to a tenth day in the method of culturing cells capable of producing desired proteins (see paragraphs [0019], [0022] and [0026]). The shortened period of time claimed necessitated only one selection from a single list of possibilities.

The reference in the patent application to "a" -indefinite article- or "the" -definite article- medium would have been read by the skilled person as one single medium, as there was no disclosure throughout the patent application of a method using multiple different fed-batch media with different amino acid compositions, whereas the working examples 1 to 3 pointed to and used a single fed-batch medium comprising serine, tyrosine and cysteine.

The basis for feature (b) of the method of claim 1 was derivable from the preferred embodiments specified in claims 11 to 13 of the patent application.

Paragraph [0015] related to an animal cell culture medium comprising at least 1 mM of serine, preferably 2 mM which is adjusted and maintained by addition of a fed-batch medium, while paragraph [0017] described that an animal cell culture medium comprised at least 1 mM of serine, and at least 1 mM of tyrosine, and/or 0.4 mM

of cysteine whose concentration in the culture solution was adjusted and maintained by addition of a fed-batch medium at least during a certain period from or after the onset of the cell growth phase. The concentrations of serine, tyrosine and/or cysteine were maintained in the culture solution at least during a part of or an entire culture period from the third day of the culture, in particular to the tenth day of the culture (see paragraphs [0024],[0027]). In case the method of culturing cells employed the fed-batch culture, serine and tyrosine and/or cysteine were dissolved at high concentrations to be enriched in a fed-batch medium and added during the culture so that the concentrations of these amino acids were maintained at predetermined concentrations or higher (see paragraph [0042]). It was clear from these paragraphs and from the working examples that all the amino acids of interest were added to the culture solution by addition of a single fed-batch medium.

The preferred embodiments set out in claims 12 and 13 defined that the "at least a part of or an entire culture period from the third day to the tenth day of the culture" referred to a culture period from the fourth, fifth, sixth, or seventh day of the culture (see paragraph [0028]). Thus, the amended period in claim 1 of "at least a part of or an entire culture period from the fourth day to the tenth day of the culture" resulted from the selection from one single list. The concentration of serine (and tyrosine and/or cysteine) in the culture solution at a predetermined concentration or higher extended furthermore preferably during a period from at least 4 days after (normally on day 5 of culture) the start point of the cell growth phase (normally around day 1 of culture) until the tenth day of the culture. In case the culture period

was no longer than two weeks (14 days), the maintenance was preferably until 4 days before the end of the culture (10 days = 14-4 days), while if it was longer than two weeks it was preferably maintained until the tenth day of the culture (see paragraphs [0022], [0024], [0026]).

The patent application did not disclose different periods of time in which the concentrations of the three amino acids are maintained.

The remaining features, such as the CHO cells, the fed-batch culture, the fed-batch medium were disclosed in claims 20, 19, 16, 15, 13 to 11 and paragraph [0036].

X. The substantive submissions made by **respondents I to V**, insofar as relevant to the present decision, may be summarized as follows:

There was no basis for the following features:

(a) "adding a fed-batch medium comprising serine, cysteine and tyrosine",
(b) "wherein the concentration of cysteine in the culture solution is maintained at 0.4 mM or higher" and
(c) "maintaining the concentration of serine, tyrosine and cysteine during a period from a fourth day to a tenth day of the culture by addition of the fed-batch medium".

Respondent I argued that in the case of a culture period being "not longer than two weeks", the end of the culture was preferably 4 days before the end of the culture, but even more preferably until 3 days before the end of the culture (see paragraph [0026] of the

patent application). Thus, the paragraph disclosed a culture period preferably until the ninth day.

Respondent II asserted that feature (a) had no basis in claim 16 of the patent application. The method of claim 16 did not specify that the amino acids had to be added into the culture solution through a fed-batch medium and were comprised in said fed-batch medium. Claim 15 was directed at a method using a fed-batch culture, wherein the components such as a carbon source, amino acids were fed into the culture solution continuously or in multiple batches sequentially. There was no disclosure in claim 15 of a fed-batch culture adding a specific set of amino acids comprised in a fed-batch medium into the culture solution. The patent application confirmed that the addition of amino acids via a fed-batch medium was not the sole option as specific components could also be added (see paragraph [0036]). The fed-batch medium of claim 24 comprising serine, tyrosine and cysteine was nonetheless limited by the specific serine concentration ranges set out in claims 21 and 22.

Paragraphs [0016] and [0024] of the patent application could not provide a basis for feature (b) because the concentration of cysteine was not maintained in the culture solution by addition of a fed-batch medium, nor was it achieved by addition of a fed-batch medium during a specific period from the fourth day to the tenth day of the culture. Even if the method of the invention described in [0017] of the patent application was directed at maintaining, by addition of a fed-batch medium, the concentrations of serine, tyrosine and cysteine in a culture solution, the serine was maintained at 1 mM and not at 2 mM as claimed in claim 1.

Although Example 3 could apparently support the method of claim 1, on closer inspection example 3 did not. First, the composition of the initial medium and the concentration of cysteine in the initial medium was unknown. Secondly, it was unknown whether the concentration of cysteine was maintained from the fourth to the tenth day and whether the pH of 1.5, instead of 1.0, and/or the CHO cell line transformed with a taurine transporter gene were not essential to achieve the effect underlying the method of culturing a CHO cell of claim 1.

Respondent III asserted that a fed-batch medium comprising serine, tyrosine and/or cysteine might contain a high concentration of serine and tyrosine and/or cysteine (see paragraphs [0036], [0038] and [0042] of the patent application). This option was nonetheless missing in claim 1.

The methods of claims 11 to 13 specified that serine and further tyrosine and/or cysteine in the culture medium were maintained at concentrations of 1 mM or 2 mM; 1mM and/or 0.4 mM or higher respectively, at least during a part of or an entire period from the third day to the tenth day of the culture. There was no direct and unambiguous disclosure for a method wherein the serine, tyrosine and cysteine were all maintained in the culture solution at the specific concentrations. Although the maintenance of a concentration of 2 mM of serine is preferred, the concentration of 0.4 mM of cysteine is not (see paragraphs [0027], [0029]). The maintenance of the concentration of serine, cysteine and tyrosine in the culture solution is ensured by a control that allows the concentration of the amino acids to be adjusted (see paragraph [0038]). The

selection of maintaining the concentration of serine, cysteine and tyrosine in the culture solution from day 4 to 10 was a threefold selection and nowhere disclosed in combination with the lower concentration threshold defined in claim 1 (see paragraphs [0022], [0028]). The period over which the concentration of serine, cysteine and tyrosine in the culture solution was maintained was selected for each amino acid separately. The maintenance of a concentration of serine, tyrosine and/or cysteine from a fourth day to a tenth day therefore also consisted of a triple selection (see paragraph [0038]).

Hence, there was no direct and unambiguous basis for a method wherein the concentration of serine was maintained at a concentration of 2 mM or higher, the concentration of tyrosine was maintained at a concentration of 1 mM or higher, and the concentration of cysteine was maintained at a concentration of 0.4 mM or higher during the fourth to tenth day of culture. There was no pointer in the patent application neither in the working example 3 nor in Figure 8 for the specific combination of features of claim 1.

Respondent IV agreed with the objections raised by respondents II and III under Article 123(2) EPC, particularly that there was no disclosure on the patent application for the multiple selections required by claim 1. Paragraph [0015] referred to the maintenance of serine only at a preferred concentration of 2 mM in the culture medium by addition of a fed-batch medium. Paragraphs [0032] and [0038] of the patent application provided no disclosure of a fed-batch medium comprising the amino acids serine, tyrosine and cysteine which had to be added to maintain a serine, tyrosine, cysteine concentration in a culture solution. Even if the

skilled person would refer to paragraphs [0042] and/or [0017] of the patent application, it referred to the addition of "a", i.e. any and not a single, fed-batch medium.
Although the cysteine in feature (b) was maintained at a concentration of 0.4 mM in the culture solution, there is no direct and unambiguous disclosure that this had to occur by means of the addition of a fed-batch medium comprising serine, tyrosine and cysteine, let alone for a shortened period of time from the 4th to 10th day of culture (see paragraphs [0024],[0027], claims 11 and 12). Paragraphs [0022] or [0026] of the patent application mentioned that it was preferable to maintain the concentration of serine (and tyrosine and/or cysteine) in the culture solution at a predetermined concentration or higher during a period from at least 4 days after (normally on day 5 of culture) the start point of the cell growth phase (normally around day 1 of culture). A culture period which was not longer than two weeks cannot be limited to 14 days with the consequence that the concentration of serine, tyrosine and/or cysteine maintained until 4 days before the end of the culture cannot be reduced to 10 days. The same rationale was applicable to a culture period which was longer than two weeks with the consequence that the concentration of serine, tyrosine and/or cysteine maintained until 3 days before the end of the culture cannot be reduced to 10 days. Hence, there was no pointer in the patent application for maintaining a specific concentration of serine, tyrosine and cysteine in the culture solution specifically during a period from at least a fourth day to the tenth day of the culture according to claim 1.

As regards the step of maintaining the concentration of serine, tyrosine and cysteine during a period from a

fourth day to a tenth day of the culture by addition of the fed-batch medium, there was no clear and unambiguous disclosure of the end date in the definition provided in paragraph [0028] of the patent application.

Respondent V argued that there was no pointer neither in the patent application in example 3, paragraph [0070], nor in paragraph [0036], claim 13, Figure 8, for maintaining the concentration of serine, tyrosine and cysteine by addition of the fed-batch medium during a period from a fourth day to the tenth day of the culture.

XI. The appellant (patent proprietor) requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request or alternatively on the basis of auxiliary requests 1 to 3 filed with its statement of grounds of appeal.

XII. The respondents I to V (Opponents 1 to 5) requested that the appeal be dismissed. They all requested further that auxiliary requests 2 to 3 not be admitted into the proceedings.

Reasons for the Decision

Main request (claims 1-5 claims)
Article 123(2) EPC

1. The main request is identical to auxiliary request 13 of the decision under appeal.

1.1 In the decision under appeal, the method of claim 1 was held to comply with Article 123(2) and (3) EPC but to offend against Article 56 EPC.

1.2 Appellant agreed with the findings of the opposition division under Article 123(2) EPC.

1.3 Respondents contended that the method according to claim 1 contravened the requirements of Article 123(2) EPC. There was no disclosure of the features

(a) "adding a fed-batch medium comprising serine, cysteine and tyrosine",
(b) "wherein the concentration of cysteine in the culture solution is maintained at 0.4 mM or higher" and
(c) "maintaining the concentration of serine, tyrosine and cysteine during a period from a fourth day to a tenth day of the culture by addition of the fed-batch medium".

2. A central question to be answered when assessing whether the amendment complies with Article 123(2) EPC is whether the combination of features of claim 1 of the main request, can be seen by the skilled person as directly and unambiguously derivable from the content as a whole of the parent application as filed. In this respect the notion of selection from two lists provides valuable guidance in the application of the so-called gold standard (see the landmark decision T 12/81 and the Case Law of the Boards of Appeal, 9th edition, 2019, II.E.16.2).

2.1 It is undisputed that the patent application does not explicitly disclose the method of claim 1 combining the features (a), (b) and (c) cited above. Thus, it remains to be assessed whether the claimed subject matter is implicitly, yet directly and unambiguously, disclosed.

2.2 An implicit disclosure means a disclosure which any person skilled in the art would objectively consider as necessarily implied in the explicit content of the patent application as a whole. It means nothing more than the clear and unambiguous consequence of what is explicitly mentioned (see e.g. T 947/05 of 27.11.2007, reasons 2.1 and T 860/00 of 28.09.2004, reasons 1.1).

Feature (a): "adding a fed-batch medium comprising serine, cysteine and tyrosine"

2.3 The decision under appeal considered that claim 16 referring back to claim 15, reading "wherein the serine and tyrosine and/or cysteine are fed into the culture solution in multiple batches sequentially or continuously", formed a basis for the feature (a) in claim 1 because "fed ... in multiple batches" implied that a fed-batch medium containing the listed amino acids had to be added.
The only difference was that the conjunction "and/or" used in claim 16 was replaced by an "and" in the present claim 1. From the fed-batch medium defined in claim 24, comprising serine as well as cysteine and tyrosine, referring back to claims 21 or 22, the skilled person could directly and unambiguously derive from the conjunction "and/or" that the embodiment including all three amino acids was preferred.

2.4 The board agrees that in the patent application claim 16 depends on claim 15 and that the "and/or" in claim 16 covers also one embodiment comprising all three amino acids.

2.4.1 However, the board cannot concur with the reasoning in point 2.1.2 of the decision under appeal that "wherein the serine and tyrosine and/or cysteine <u>are fed</u> into

the culture solution in multiple batches sequentially or continuously" (claim 16, emphasis added) necessarily implies that the fed-batch culture comprises a fed-batch medium comprising all of serine, tyrosine and cysteine.
Claims 15 or 16 do not specify that the fed-batch culture comprised the addition of a specific set of amino acids consisting of serine, tyrosine and cysteine into the culture solution by means of a fed-batch medium. The addition of amino acids via a fed-batch medium is not the only option, as also "only specific components may be added" (see paragraph [0036] of the patent application). The wording of claims 15 and 16 does not exclude the feeding of the amino acids serine, tyrosine and/or cysteine as components, even separately from each other, and not in a fed-batch medium.
Finally, the fed-batch medium comprising serine, tyrosine and cysteine according to claim 24 required the concentration of serine in the fed-batch medium to range between 10 mM to 1000 mM or 20 mM to 500 mM (see claims 21 and 22), which is absent in the present claim 1.

2.4.2 Thus, even if the method according to claim 25 of the patent application is taken into account, there is no direct and unambiguous disclosure of a method as defined in present claim 1, comprising the step of adding a fed-batch medium comprising serine, tyrosine and cysteine, wherein serine is present at any concentration, but nonetheless sufficient to maintain the serine concentration in the culture solution above a specific threshold concentration level.

"[M]aintaining the concentration of serine, cysteine and tyrosine in the culture medium by addition of the fed-batch medium"

2.5 The decision under appeal considered that paragraphs [0038] and [0039] of the patent application provide a basis for the maintenance of the concentration of all three amino acids in the culture solution.

2.6 The board agrees that in case of employing the fed-batch culture, the medium that is to be added during the culture may contain a high concentration of serine (and tyrosine and/or cysteine). It is important "to maintain the concentration of serine, or respective concentrations of serine and tyrosine, or respective concentrations of serine and cysteine, or respective concentrations of serine, tyrosine, and cysteine, at a predetermined concentration or higher at least during a predetermined stage of the culture, as described above." (see paragraph [0038] of the patent application).

The maintenance of the concentration of serine (and tyrosine and/or cysteine) in the culture solution at a predetermined concentration can be achieved either by addition of a high concentration of serine (and tyrosine and/or cysteine) to the medium at an early stage of the cell culture, or alternatively by addition of a medium comprising a high concentration of serine (and tyrosine and/or cysteine) during the culture to supplement the medium with amino acids (see paragraph [0031] of the patent application).
It is only the maintenance of the concentration of serine by adding fed-batch medium in the culture solution that finds a basis in paragraph [0015] of the patent application.

2.6.1 Although the addition of "a" fed-batch medium containing a high concentration of serine (and tyrosine

and/or cysteine) for maintaining the concentration of serine, tyrosine and/or cysteine in the culture solution is directly and unambiguously derivable from paragraphs [0031], [0038] and [0042] of the patent application, the board cannot derive therefrom that the different serine, tyrosine and cysteine concentrations in the culture solution should be maintained by the addition of a single fed-batch medium comprising serine, tyrosine and cysteine.

2.7 Even if the language of claims 15 and 16 is read in the light of the patent application as a whole, the board cannot share appellant's conclusion that the reference in the patent application to "a" -indefinite article- or "the" -definite article- medium would be read by the skilled person as one single medium, i.e. a single fed-batch medium comprising serine, tyrosine and cysteine.

2.7.1 The burden of demonstrating that the method according to claim 1 has a basis in the patent application rests with the appellant (i.e. proprietor). The indication that a method, which uses several different fed-batch media, has no basis in the patent application is insufficient to establish that the method of claim 1 using a single fed-batch medium complies with Article 123(2) EPC.

2.7.2 Firstly, [i]n the fed-batch culture, a (indefinite article) medium is fed continuously or sequentially during cultivation [...] (see paragraph [0036] of the patent application). The medium to be added in the fed-batch culture is referred to as "fed-batch medium" and does not necessarily have to be the same medium as that used in the culture (hereinafter "initial medium"); namely a different medium may be added, or only specific components may be added.

2.7.3 Secondly, the medium that is to be added during the culture may contain a high concentration of serine (and tyrosine and/or cysteine) (see paragraph [0038] of the patent application). In the fed-batch culture, it is important "to maintain the concentration of serine, or respective concentrations of serine and tyrosine, or respective concentrations of serine and cysteine, or respective concentrations of serine, tyrosine, and cysteine, at a predetermined concentration or higher at least during a predetermined stage of the culture". " ... the concentration of serine (and tyrosine and/or cysteine) in the culture solution may be monitored to adjust the concentrations of these amino acids in the medium to be added (i.e. fed-batch medium) so that the concentrations of these amino acids in the culture solution can be controlled".

2.7.4 From paragraphs [0036] and [0038], it cannot be directly and unambiguously derived that the medium to be added during the fed-batch culture must be one single fed-batch medium, just because the medium to be added must be different from the initial medium or because only specific components may be added. "[T]he" fed-batch medium is a medium which is fed during fed-batch culture. "The" fed-batch medium refers to the type of medium fed during culture, not to the composition of the medium itself. The fact that the concentration of serine (and tyrosine and/or cysteine) in the medium to be added (i.e. fed-batch medium) may be adjusted so that the concentrations of these amino acids in the culture solution are maintained at or above the desired concentration threshold, confirms this view and does not directly and unambiguously establish that one single fed-batch medium is used in the fed-batch culture of the invention.

2.8 In addition, paragraph [0042] of the patent application mentions that in case of a fed-batch culture, serine and tyrosine and/or cysteine are dissolved at high concentrations to be enriched in a fed-batch medium and a fed-batch medium is added either continuously or sequentially during cultivation so that the concentrations of these amino acids are maintained at predetermined or higher concentrations.

2.8.1 The use of the conjunction "and/or" to define the presence of a high concentration of serine (and tyrosine and/or cysteine) in the feed batch medium or that these high concentrations of amino acids may be contained in the culture medium from an initial stage of the cell culture in paragraphs [0017], [0038] and [0042] do not directly and unambiguously disclose that the fed-batch culture uses "a" single fed-batch medium containing all of the amino acids that are to be maintained in the culture solution.

2.8.2 Thus, neither the preparation of "a" fed-batch medium nor the addition of "a" medium to maintain the concentration of desired amino acids at or above a concentration threshold in the culture solution teaches directly and unambiguously the use of one single fed-batch medium, even less of one single fed-batch medium containing all the amino acids to be maintained.

2.8.3 The skilled person would have understood that the method according to claim 1 is at most reproduced in Example 3 among all the examples. In this example the concentration of serine and tyrosine in the culture solution was maintained at or above the claimed concentration threshold while no indication is given as to whether the concentration of cysteine was maintained

or not. Even if a fed-batch medium comprising serine, cysteine and tyrosine at high specific concentrations was used in example 3, it neither directly nor unambiguously discloses the use of one single fed batch medium comprising any concentration of serine, tyrosine and cysteine in a fed batch culture for maintaining the concentration of the serine and tyrosine and cysteine at or above the claimed concentration threshold in the culture solution.

Feature (b):"[W]herein the concentration of cysteine in the culture solution is maintained at 0.4 mM or higher".

2.9 The decision under appeal considered that paragraphs [0016] and [0024] of the patent application provide explicit basis for "concentration of cysteine in the culture solution may be 0.4 mM or higher". Paragraph [0027] also states that "the concentration of cysteine in the culture solution may be maintained at 0.4 mM or higher".

2.9.1 Appellant indicated that the "concentration of cysteine is maintained at 0.4 mM or higher" was based on claim 12 and paragraphs [0017], [0024] and [0027] of the patent application.

2.10 The board agrees that claim 12 referring back to claim 11 relates to a method of culturing a cell, further characterized in that a concentration of tyrosine in the culture solution is maintained at 1 mM or higher, and/or a concentration of cysteine in the culture solution is maintained at a concentration of the cysteine in an initial medium or higher or at 0.4 mM or higher at least during a part of or an entire period from the third day to the tenth day of the culture.

2.10.1 The board agrees that an animal cell culture medium comprising 1 mM or higher of serine or a salt thereof, and at least 1 mM or higher of tyrosine and/or 0.4 mM or higher of cysteine is disclosed (see paragraph [0017] of the patent application). This medium refers not only to a medium comprising cysteine at a concentration of 0.4 mM or higher in an initial medium but also a medium adjusted such that a concentration of cysteine in the culture solution is maintained at 0.4 mM or higher by addition of a fed-batch medium or the like at least during a certain period from or after the onset of the cell growth phase. Since a typical concentration of cysteine in the initial medium is about 0.4 mM, the concentration of cysteine in the culture solution may be 0.4 mM or higher, preferably 1 mM or higher, during a part of or an entire culture period from the third day of the culture, regardless of the concentration of cysteine in the initial medium (see paragraphs [0024] and [0027] of the patent application). Thus, feature (b) has a basis in the patent application.

Feature (c): "[M]aintaining the concentration of serine, cysteine and tyrosine during a period from a fourth day to a tenth day of the culture by addition of the fed-batch medium."

2.11 Respondents argued that the selection of the sub-period of maintenance of the concentration of serine, cysteine and tyrosine in the culture solution from day 4 to 10 was a threefold selection -for each amino acid separately- which was nowhere disclosed in combination with the lower concentration threshold defined in claim 1 (see paragraphs [0022], [0028], [0038]).
Moreover, Example 3 could not really support the method of claim 1.

2.12 Appellant asserted that feature (c) of claim 1 was based on claims 12, 13 and paragraphs [0027] to [0029] of the patent application. This concept was further elaborated in paragraphs [0019], [0022] and [0026]).

2.13 The board concurs with appellant's view. Claims 12 and 13 of the patent application disclose a method of culturing a cell to produce a desired protein, characterized in that a concentration of serine in a culture solution is maintained at 2 mM or higher at least during a part of or an entire period from the third day to the tenth day of the culture. Further, a concentration of tyrosine in the culture solution is maintained at 1 mM or higher, and/or a concentration of cysteine in the culture solution is maintained at a concentration of the cysteine in an initial medium or higher or at 0.4 mM or higher at least during a part of or an entire period from the third day to the tenth day of the culture (emphasis added).
The highlighted expression stands for a culture period from the fourth, fifth, sixth, or seventh day of the culture, or a culture period from the start point of the culture or from the first or second day of the culture including a part of or an entire culture period from the third day to the tenth day of the culture (see paragraph [0028] of the patent application). Hence, the period of time combining a starting point of this period "from a fourth day" with the end-point "to a tenth day" has a direct and unambiguous basis in this paragraph. The now claimed shortened time span necessitates only one selection from a single list of equal possibilities.

Combination of features recited in claim 1

2.14 The board, in applying the so-called gold standard (see point 2. of the reasons above), notes that there is neither an explicit nor an implicit yet direct and unambiguous disclosure in the patent application of a method combining the features (a) to (c) as in claim 1: wherein all of serine, tyrosine and cysteine are selected and maintained at different concentrations: 2 mM, 1 mM and 0.4 mM, respectively, each of them from the/a fourth to the/a tenth day of the culture, by means of an addition of the fed-batch medium comprising all the three amino acids.

2.14.1 Since a fed-batch medium for use in a fed-batch culture method is defined in the patent application as a medium comprising serine and tyrosine and/or cysteine, i.e. comprising either serine and tyrosine; serine and cysteine; or serine, tyrosine and cysteine (see claim 16; paragraphs [0042] and [0038]), the skilled person has to first select a fed-batch medium comprising the combination of serine, tyrosine and cysteine from the three alternatives described above.

2.14.2 There is no pointer to a fed-batch medium having serine, tyrosine and cysteine, neither in the claims or paragraphs [0038] and [0042], nor in example 3 of the patent application. Each of the fed-batch media is equally preferred and suitable for fed-batch culture.

2.14.3 The board notes that Example 3 does not exactly reproduce the method of claim 1. Although the addition of fed-batch medium comprising serine, tyrosine and cysteine is more effective than fed-batch media containing only some of them, in fed-batch culture they all achieve a better protein yield than the control fed-batch culture. Again, based on example 3, there is neither a pointer nor a direct and unambiguous

disclosure that the use of the claimed fed-batch medium comprising serine, tyrosine and cysteine would be preferable to any other disclosed fed-batch media.

2.15 Claims 11 to 13 disclose methods of culturing cells comprising maintaining the concentrations of serine, tyrosine and/or cysteine in the culture solution. Claim 13 refers back to claims 11 or 12. Claim 13 relates to an embodiment where serine is maintained at 2 mM in the culture solution. Claim 12 specifies that tyrosine is maintained at 1 mM and/or that cysteine is maintained at a concentration of an initial medium or higher or at 0.4 mM or higher in the culture solution respectively. Claim 11 requires maintenance of the serine concentration at 1 mM or higher.

Thus, claims 11 to 13 disclose the following combinations of amino acid concentrations (or higher) to be maintained: 1) Ser 1 mM (claim 11); 2) Ser 2 mM (claim 13 referring to claim 11); 3) Ser 1 mM, Tyr 1mM (claim 12); 4) Ser 1 mM, Cys initial concentration (claim 12); 5) Ser 1 mM, Cys 0.4 mM (claim 12); 6) Ser 1mM, Tyr 1mM, Cys initial concentration (claim 12); 7) Ser 1mM, Tyr 1mM, Cys 0.4 mM (claim 12); 8) - 12) combinations 3) to 7) but with 2 mM Ser (claim 13 referring to claim 12).

All these combinations of features are equally preferred without any pointer to a particular combination.

2.15.1 The skilled person has to select a particular combination from this list to specifically maintain serine, tyrosine and cysteine at a concentration at or above 2 mM, 1 mM and 0.4 mM, respectively in the culture solution. This constitutes a second selection,

for which there is neither a pointer nor a direct and unambiguous disclosure thereof. Indeed, the adjusted medium comprising 1 mM or higher of serine or a salt thereof, and at least 1 mM or higher of tyrosine and/or 0.4 mM or higher of cysteine in paragraphs [0017], [0042] or examples 1 to 3 of the patent application fail to disclose the maintenance of serine, tyrosine and cysteine in the culture solution at or above 2 mM, 1 mM and 0.4 mM respectively in combination.

2.16 Finally, the method of culturing cells according to claims 11 to 13 of the patent application specifies that a concentration of serine, tyrosine and/or cysteine is maintained at least during a part of or an entire period from a third day to a tenth day of the culture. The expression "at least during a part of or an entire period from a third day to a tenth day of the culture" is further defined in paragraphs [0022] and [0028] and refers to a culture period from the fourth, fifth, sixth, or seventh day of the culture or a culture period from the start point of the culture or from the first or second day of the culture including a part of or an entire culture period from the third day to the tenth day of the culture.
The skilled person has to select from the definition provided in paragraph [0028] that the culture period is shortened from the fourth day to a tenth day of culture among all the equal possibilities to arrive at the method of claim 1. This constitutes a third selection from a list for which there is neither a pointer nor a direct and unambiguous disclosure thereof.

2.16.1 In view of the above considerations, the skilled person has to select the claimed features from three alternatives and two different lists of a certain length and equally weighted possibilities in the

application as filed to arrive at the combination of features of the method according to claim 1. A method with such a combination of features for which there is no clear pointer extends beyond the content of the application as filed (see e.g T 686/99 of 22 January 2003, reasons 4.3.3).

2.17 The board therefore concludes that the method of claim 1 contravenes Article 123(2) EPC.

Admission of auxiliary requests 2 and 3 filed with appellant's statement of grounds of appeal into the proceedings.

3. Auxiliary requests 2 and 3 were first filed with the statement of grounds of appeal.

4. The respondents requested not to admit them into the proceedings.

5. Pursuant to Article 12(4) RPBA 2007, which applies to the present case under Article 25(2) RPBA 2020, the board has a discretion to hold inadmissible facts, evidence or requests, which could have been presented or were not admitted into the first instance proceedings.

6. Auxiliary requests 2 and 3 derive from the main request and auxiliary request 1, respectively, by combination of claims 1 to 4. Formally, the amendment corresponds to the deletion of claims 1 to 3 while retaining claim 4. The deletion of the subject-matter defined in claims 1 to 3 from the main and auxiliary request 1 cannot have taken the respondents by surprise as the proposed amendments do not change the legal and factual scope of the proceedings. Claim 4 was already present in

auxiliary requests 13 and 14 in the opposition proceedings.

6.1 Since the above deletion neither creates a fresh case nor affects procedural economy, the board, availing itself of its discretionary power under Article 12(4) RPBA 2007, decided to admit the new auxiliary requests 2 and 3 into the appeal proceedings.

Auxiliary requests 1 to 3
Article 123(2) EPC

7. The findings on Article 123(2) EPC in connection with claim 1 of the main request (see paragraphs 2.4 to 2.17 above) equally apply to claim 1 of each of the auxiliary requests 1 to 3 in that they combine the same technical features as claim 1 of the main request.

8. Since no allowable request is on file, the appeal must be dismissed.

Order

For these reasons it is decided that:

1. The appeal is dismissed.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated