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Datasheet for the decision of 11 June 2013

Case Number: T 2244/09 - 3.3.08

Application Number: 02767141.1

Publication Number: 1417301

IPC: C12N 9/00, C12N 9/24, C12N 9/96

Language of the proceedings: EN

Title of invention:
Carbohydrates and polyols for dissolving protein crystals

Patent Proprietors:
Novozymes A/S
Novozymes North America, Inc.

Opponent:
Danisco US, Inc.

Headword:
Enzyme Solubilization/NOVOZYMES

Relevant legal provisions:
EPC Art. 54, 56
RPBA Art. 13(1)

Keyword:
"Admissibility of new documents and Auxiliary Requests (yes)"
"Main Request and Auxiliary Requests 1 to 8 - novelty (no)"
"Auxiliary Requests 9 to 11 - inventive step (no)"

Decisions cited:
T 0190/99, T 0423/01, T 0870/02

Catchword:
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Case Number: T 2244/09 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 11 June 2013

Appellant: (Opponent)
Danisco US Inc.
925 Page Mill Road
Palo Alto CA 94304—1013 (US)

Representative:
Armitage, Ian Michael
Mewburn Ellis LLP
33 Gutter Lane
London EC2V 8AS (GB)

Respondents: (Patent Proprietors)
Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsvaerd (DK)

Novozymes North America, Inc.
77 Perry Chapel Church Road
Franklinton, NC 27525 (US)

Representative:
Stevens, Ian Edward
Potter Clarkson LLP
The Belgrave Centre
Talbot Street
Nottingham NG1 5GG (GB)

Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 11 September 2009 rejecting the opposition filed against European patent No. 1417301 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman: T. J. H. Mennessier
Members: P. Julià
D. S. Rogers

Summary of Facts and Submissions

I. European patent 1 417 301 was opposed on the grounds of Articles 100(a) EPC (Articles 54 and 56 EPC) and 100(b) EPC (Article 83 EPC). The opposition division considered the granted claims to fulfil the requirements of the EPC and, accordingly, rejected the opposition.

II. The opponent (appellant) lodged an appeal and, on 21 January 2010, filed the statement setting out its grounds of appeal. With a letter dated 12 March 2010, the appellant requested the board to exercise its discretion and to admit documents D9 to D11 into the appeal proceedings.

III. On 17 May 2010, the patentees (respondents) replied to the appellant's statement of grounds of appeal and filed auxiliary requests 1 to 7 and document D12.

IV. On 25 August 2010, the appellant replied to the respondents' letter and requested the board to exercise its discretion and to admit document D13 into the appeal proceedings.

V. On 6 October 2010, the respondents requested the board not to admit document D13 into the appeal proceedings and auxiliary requests 8 to 11 were filed in the event that this request was not allowed.

VI. With letter dated 26 January 2011, the appellant requested that document D13 be admitted into the proceedings.

VII. On 11 February 2013, the board sent a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) informing the parties of its preliminary, non-binding opinion on some issues of the case, in particular, its positive opinion on the admissibility of the new documents and auxiliary requests as well as on the interpretation of the claims, and the relevance of document D13 as regards novelty and inventive step (as possible closest prior art document), if this document was admitted into the appeal proceedings.

VIII. No further substantive submissions were filed by the respondents which, with a letter dated 12 April 2013, informed the board of their intention not to attend the oral proceedings.

IX. On 10 May 2013, further substantive submissions were filed by the appellant which informed the board of its intention to attend the scheduled oral proceedings.

X. Oral proceedings took place on 11 June 2013 in the absence of the respondents. At the end of the proceedings, the Chairman announced the decision of the board.

XI. The respondents' **main request** (granted claims) contained 14 claims. Claim 1 read as follows:

"1. A method for recovering a protein of interest from a culture solution comprising a recovery step in which step the protein of interest is in solution but above its solubility limit, comprising adding a carbohydrate

and/or a polyol to the culture solution immediately after said recovery step."

Claims 2 to 14 were directed to preferred embodiments of claim 1.

XII. Claim 1 of **auxiliary request 1** read as claim 1 of the main request except for the presence of the additional sentence "*without having the protein of interest in precipitated form*" after the reference to the protein of interest being "*in solution but above its solubility limit*".

XIII. Claim 1 of **auxiliary request 2** read as claim 1 of the main request except for the fact that the protein of interest was required to be "*fully dissolved*".

XIV. Claim 1 of **auxiliary requests 3, 4 and 5** read as claim 1 of the main request and of auxiliary requests 1 and 2, respectively, with the introduction of the additional feature "*wherein the carbohydrate and/or the polyol is added immediately after a recovery concentration unit step*".

XV. Claim 1 of **auxiliary request 6** read as follows:

"1. A method for recovering a protein of interest from a culture solution comprising a recovery step in which step the protein of interest is concentrated to form a concentrate in which the protein of interest is in solution but above its solubility limit, comprising adding a carbohydrate and/or a polyol to the concentrate immediately after said recovery step".

XVI. Claim 1 of **auxiliary request 7** read as claim 1 of auxiliary request 6 except that the protein of interest was required to be *"concentrated in a continuous mode*".

XVII. Claim 1 of **auxiliary request 8** read as claim 1 of auxiliary request 3 except for the deletion of the terms "*a carbohydrate and/or*" and "*the carbohydrate and/or*".

XVIII. Claim 1 of **auxiliary request 9** read as claim 1 of auxiliary request 8 except for the sentence "*and the polyol is selected from those having the general formula $C_nH_{2n+2}O_n$, wherein n is from 4 to 8, glycerol and monopropylene glycol*" introduced at the end of the claim.

XIX. Claim 1 of **auxiliary request 10** read as claim 1 of auxiliary request 9 except that it was specified that the "n" of the general formula had a value "*from 4 to 6*".

XX. Claim 1 of **auxiliary request 11** read as claim 1 of auxiliary request 8 except for the sentence "*and the polyol is selected from the group consisting of sorbitol, mannitol, erythritol, ribitol, xylitol, glycerol and monopropylene glycol*" introduced at the end of the claim.

XXI. The documents cited in the present decision are:

D3: EP 0 201 017 (publication date: 12 November 1986);

D4: US 3,717,550 (publication date: 20 February 1973);

D5: C. Russell Middaugh and David B. Volkin, "Chapter 4: Protein Solubility", pages 109 and 120 to 129. In T.J. Ahern and M.C. Manning eds., "Stability of Protein Pharmaceuticals", 1st ed., 1992, New York;

D9: Declaration of Dr Alfred Gaertner, signed 10 March 2010;

D10: S.N. Timasheff, Annu. Rev. Biophys. Biomol. Struct., 1993, Vol. 22, pages 67 to 97;

D11: C.H. Schein, Bio/Technology, April 1990, Vol. 8, pages 308 to 317;

D13: US 4,724,208 (publication date: 9 February 1988).

XXII. Appellant's arguments, as far as relevant for the present decision, may be summarized as follows:

Admissibility of new documents and auxiliary requests

Document D13 was brief, simple and, *prima facie*, highly relevant. The contents of document D13 had been considered by the respondent which, in reply thereto, had filed auxiliary requests that took into account the disclosure of this document.

Main request and auxiliary requests
Interpretation of the claims

In line with the case law, claim 1 had to be broadly interpreted. Features that were not in the claim could not be relied on to argue in favour of novelty or inventive step. The wording of claim 1 did not require

the carbohydrate and/or polyol (C/P) to be added to a supersaturated solution, the presence of precipitate with the remaining solution (being either supersaturated or not supersaturated) was not excluded, and, indeed, it did not require the resulting solution, i.e. with added C/P, to be supersaturated.

Main request and auxiliary requests 1 to 8
Article 100(a) EPC; Article 54 EPC

Document D13 was concerned with the provision of enzyme solutions at high concentrations by adding a starch (carbohydrate/polyol) to a culture solution, inhibiting thereby enzyme agglomeration and increasing enzyme solubility. Methods were disclosed that did not involve enzyme precipitation and had the features of the claimed method. Nothing in document D13 suggested that these methods were unimportant or that they should not be used. Indeed, they were supported by the examples, such as in Example II, and described as preferred embodiments. The advantages of the claimed method, to the extent that they existed at all, were advantages of the method of document D13.

None of the features introduced into auxiliary requests 1 to 8 (AR1 to AR8) differentiated the claimed subject-matter from the method disclosed in document D13. According to Example II of this document, a precipitate appeared after stirring the solution for 40 hours and then centrifuging. There was no mention of a precipitated enzyme before these steps (AR1) and thus, the enzyme was fully dissolved (AR2). Ultrafiltration and evaporation were recovery concentration steps (AR3). The combination of these features were found in the

method disclosed in document D13 (AR4 and AR5). Both recovery steps resulted in a concentrate enzyme form to which the starch was added (AR6) and the recovery steps were performed in a continuous mode (AR7). Moreover, carbohydrates were polyols and thus, a broad interpretation of the latter term necessarily included the former (AR8).

Auxiliary requests 9 to 11
Article 100(a) EPC; Article 54 EPC

The carbohydrate composition of the polyol/starch used in the examples of document D13 (Maltrin-100) contained 84% of pentasaccharides and thus, fell within the generic formula and the polyols of auxiliary requests 9 to 11.

Article 100(a) EPC; Article 56 EPC

The method disclosed in the closest prior art document D13 was exemplified with a polyol/starch that was a substrate of the enzyme. However, the overall teaching of this document was not limited thereto since the technical effect achieved did not rely specifically on an enzyme-substrate interaction. Rather, it was understood that the function of the polyol/starch was to inhibit the aggregation between enzyme molecules and to increase thereby the solubility of the enzyme. The effect of polyols on the solubility of enzymes/proteins was known at the priority date of the patent. As shown in the examples of the patent, the same effect was achieved by using the polyols of auxiliary requests 9 to 11. Starting from this closest prior art, the technical problem to be solved could be formulated only

in minimalist terms, namely the provision of an alternative method for reducing enzyme precipitation by using polyols other than the starch used in document D13 but achieving the same effect as this starch. As shown in documents D5 and D11, a textbook and a review article reflecting the common general knowledge, the polyols of auxiliary requests 9 to 11 were well-known in the art for the same purpose, i.e. for increasing enzyme/protein solubility. Their selection was obvious and did not require any inventive skill. This was supported by document D4, which showed the use of these polyols for increasing the solubility of the same enzyme as the one used in the method of document D13.

XXIII. Respondents' arguments submitted in writing, as far as relevant to the present decision, may be summarized as follows:

Admissibility of new documents and auxiliary requests

Article 12(2) RPBA required the statement of grounds of appeal to contain the appellant's complete case. Documents D9 to D11 were filed after the filing of the appellant's grounds of appeal and document D13 was filed even later. Document D13 was thus extremely late-filed and its content was less relevant than the content of other documents already on file. Auxiliary requests 8 to 11 were filed only in the event that document D13 was admitted into the appeal proceedings.

Main request and auxiliary requests
Interpretation of the claims

The requirement that the protein of interest was "*in solution but above its solubility limit*" had a clear meaning for a skilled person, namely that the protein was in a supersaturated state. A skilled person with a mind willing to understand would not consider claim 1 to allow the protein of interest to be in a precipitated form. Firstly, claim 1 required the protein to be in solution, i.e. dissolved, not suspended or dispersed as a solid. Secondly, claim 1 required the protein to be in solution but above its solubility limit, thereby excluding the presence of solid or precipitated protein. A protein solution in contact with a solid form of the protein was either at - but not above - the solubility limit of the protein or else undergoing precipitation or crystallisation, which was ruled out by the teachings of the patent. If, as required by claim 1, the carbohydrate and/or a polyol (C/P) was added "*immediately after*" the recovery step in which step the protein of interest was supersaturated, the C/P was then added to a supersaturated solution in line with the teachings of the patent. Any other possibilities would have been ruled out by a skilled person with a mind willing to understand.

Article 100(a) EPC; Articles 54 and 56 EPC

Document D13 dealt with the long-term stabilization of a solution of α-amylase from *B. licheniformis* but not with the recovery process itself or with the problems encountered during recovery of the enzyme. In Example I,

the storage stability of the enzyme was studied over one month. The advantages of the process disclosed in the patent were not appreciated in document D13 since it gave equal, if not greater, importance to the precipitation of the enzyme as shown by the presence of step D(i) (precipitating a cake containing the enzyme) in the method of this document. Moreover, according to document D13, the disclosed method required the formation of an enzyme/substrate complex and thus, it was necessary to add a substrate for the enzyme into the solution. In line with this disclosure, Table IV showed that non-substrate, low molecular weight polyols, such as maltose and glucose, increased the amount of precipitated enzyme. Thus, document D13 led a skilled person away from using anything other than a substrate for the enzyme and away from using low molecular weight polyols.

Even if the use of C/P for increasing the solubility of proteins/enzymes would have been known in the prior art concerned with industrial recovery of proteins from a culture broth (see, *inter alia*, documents D4 and D5) and if, for the sake of argumentation, a skilled person would have been free to use the C/P wherever and whenever needed, this did not render it obvious to take the protein/enzyme to supersaturation and then to add C/P in order to avoid an inadvertent precipitation or crystallization of this protein/enzyme, let alone in the expectation of achieving the advantages of the method of the patent (defined by the subject-matter of the claims) as acknowledged by the opposition division in the decision under appeal. The mere listing of the many factors that were known in the art to affect the solubility of a protein could not lead a skilled person

to select only one of these factors, let alone to combine it with operating the method at supersaturation.

XXIV. The appellant (opponent) requested that the decision under appeal be set aside and the patent be revoked.

XXV. The respondents (patentees) requested in writing that the appeal be dismissed, or that the patent be maintained upon the basis of any of auxiliary requests 1 to 11 filed during the course of the written procedure.

Reasons for the Decision

Admissibility of new documents and auxiliary requests

1. Document D9, a declaration of a technical expert in the field, was filed by the appellant after the filing of its statement of grounds of appeal. Documents D10 and D11, two review articles published in 1991 and 1990, respectively, were cited in this declaration and filed therewith (cf. Section II *supra*). Thus, the filing of these documents represents an amendment of the appellant's case in the sense of Article 13(1) RPBA and, accordingly, their admissibility lies within the board's discretion.

2. According to the appellant, documents D9 to D11 were filed only to reinforce its arguments regarding the common general knowledge and they did not introduce any new issues into the proceedings. These documents were filed before the respondents' reply to the appellant's grounds of appeal. The content of these documents was

addressed by the respondents in their reply and no request was made therein against their admissibility into the appeal proceedings.

3. Document D13 was filed three months after the respondents' reply to the appellant's grounds of appeal (cf. Section IV *supra*). The filing of this document represents again an amendment of the appellant's case in the sense of Article 13(1) RPBA and lies within the board's discretion to admit it into the proceedings.

4. According to Article 13(1) RPBA, the discretion shall be exercised in view of *inter alia* the complexity of the new subject matter submitted, the current state of the proceedings and the need for procedural economy. In the present case, the contents of document D13, in particular Example II upon which the appellant's argumentation relies, do not add, in the board's view, undue complexity to the case. In their submissions against the admissibility of document D13, the respondents addressed the relevance of this document and filed auxiliary requests 8 to 11 that took into account the contents of this document (cf. Section V *supra*). The *prima facie* relevance of document D13 was acknowledged by the board in its communication pursuant to Article 15(1) RPBA, in which the parties were also informed of the board's positive opinion on the admissibility of documents D13 and D9 to D11 into the appeal proceedings (cf. Section VII *supra*).

5. After the board's communication, no further substantive submissions were made by the respondents, which did not take the opportunity to attend the oral proceedings before the board and/or to put forward further

arguments against the relevance of document D13 and its admissibility into the appeal proceedings (cf. Section VIII *supra*).

6. In view of the prosecution history of the present case, the nature and content of documents D9 to D11 and the relevance of document D13, the board does not see any reason to deviate from its preliminary opinion as set out in its communication pursuant to Article 15(1) RPBA and, exercising its discretion under Article 13(1) RPBA, decides to admit these documents into the appeal proceedings. Consequently, as stated in its communication under Article 15(1) RPBA, auxiliary requests 8 to 11 are also admitted into the proceedings.

Main request and auxiliary requests
Interpretation of the claims

7. An important issue in the present appeal proceedings is the interpretation of the claims. According to the established case law, when novelty and inventive step are assessed, there is no reason to interpret an excessively broad claim more narrowly, if it is a question not of understanding concepts that require explanation but rather a question of examining an excessively broad request in relation to the state of the art (cf. "Case Law of the Boards of Appeal of the EPO", 6th edition 2010, I.C.2.9, page 105).

8. The following features in claim 1 of the main request are controversial: "*a recovery step in which step the protein of interest is in solution but above its solubility limit*" and "*adding a carbohydrate and/or a*

polyol to the culture solution immediately after said recovery step". The following issues are of relevance:

8.1 The nature of the "*recovery step*" is not defined in claim 1 of the main request and thus, it may be any of the steps defined in paragraph [0034] of the "*Recovery processing*" disclosed in the patent including the initial steps. In auxiliary requests 3 to 5 and 8 to 11 (*supra*), this step is characterized as being a "*recovery concentration unit step*" which according to the patent may involve an ultrafiltration step or an evaporation (cf. paragraph [0038], lines 52 to 54 of the patent).

8.2 The feature "*above its solubility limit*" is considered to be a relative feature. The solubility limit of a protein is not a single, discrete (absolute) value but depends on various parameters, particularly on the specific system used, such as the solvent and the given set of conditions in which the protein is found (cf. *inter alia* paragraphs [0042], [0044] and [0055] *et seq*. of the patent). The solubility limit of a protein recovered from the initial steps of the recovery processing disclosed in the patent, such as the crude culture broth, may be different from that of a protein recovered from latter steps of this processing, such as a recovery concentration step in which the protein is (almost) pure and dissolved in a defined solvent. The presence of heterogeneous solutions and of impurities may influence the solubility of the protein (cf. *inter alia* paragraph [0088], lines 25 to 27, paragraph [0089], lines 55 to 57 of the patent). Thus, contrary to the opinion of the opposition division (cf. page 4, lines 17 and 18 of the decision under appeal), a

protein has no "*intrinsic solubility*", i.e. a discrete, absolute value, but this value depends on the particular conditions given.

8.3 This interpretation is also derivable from the patent itself. Although Examples 2 and 3 of the patent do not disclose the origin of "*the non-crystallized or non-precipitated protease enzyme solution*" and refer only "*to dissolve the enzyme crystals and/or amorphous enzyme precipitate during the production of enzymes*" (cf. page 9, 24 to 25 and 54 to 55 of the patent), Examples 4 and 5 refer to the enzyme "*crystals/precipitate*" being "*observed as sludge in the concentrate*" which is obtained "*fermented as known in the art*" (cf. paragraphs [0093] and [0096] of the patent). Neither the degree of purity of the enzymes used in these examples nor their "*intrinsic solubility*" is disclosed therein and thus it cannot be derived therefrom whether the concentrates in solution are supersaturated in the sense given by the opposition division in the decision under appeal. The sole criterion for defining the solution as supersaturated is the appearance of a "*sludge*" or precipitate. It is also worth noting in this context that, according to the established case law, the same standard has to be applied when assessing the disclosure of a prior art document and that of the patent specification (cf. *inter alia* T 870/02 of 16 September 2004, point 6 of the Reasons; T 423/01 of 25 March 2004, point 21 of the Reasons).

8.4 The precipitation and crystallization of a protein, when in solution but above its solubility limit, is a dynamic, continuous process which, although being most

often rather slow (hours), can also be very fast (seconds) (cf. paragraph [0043], lines 47 to 50 of the patent). Thus, immediately after a recovery step, a protein may be at the beginning of a precipitation or crystallization process that may be, *inter alia*, either very slow or extremely fast. The board considers the wording of claim 1 of the main request to comprise possible embodiments in which the carbohydrate and/or polyol addition takes place at this moment, i.e. in the presence of an initial amount of precipitate or crystal - no matter how small it may be (in a slow precipitation or crystallization process) - or in the presence of a significant amount of precipitate or crystal (in a fast precipitation or crystallization process).

8.5 Auxiliary requests 1, 2, 4 and 5 (*supra*) address this issue by excluding the presence of protein in precipitated form in the recovery (concentration unit) step. The board, however, considers that the features introduced into these auxiliary requests do not clearly exclude the embodiments referred to above, i.e. the presence of a protein precipitate "*immediately after said recovery step*". The more so when account is taken of the fact that the wording "*immediately after*" is relative and does not set a defined time range or limit. If broadly interpreted, it may also be understood as requiring to carry out the C/P addition to the culture solution just before performing any further recovery (concentration unit) step.

9. The board is convinced that the interpretation of the claims made above is technically sensible (cf. T 190/99 of 6 March 2001).

Main request and auxiliary requests 1 to 7
Article 100(a) EPC; Article 54 EPC

10. Document D4 is the sole prior art document cited in the decision under appeal in support of objections raised under Article 54 EPC. The opposition division acknowledged the novelty of the claimed subject-matter of the main request relying mainly on the fact that, in its view, it was not unambiguously derivable from this document i) whether the protein of interest in solution was above its solubility limit, since the presence of turbidity could have many causes and not just arise from a supersaturated state of the protein, and ii) whether the addition of C/P was immediately after a recovery step, since no recovery step was identified (cf. page 4, second paragraph of the decision under appeal). The interpretation of the claims in point 8 *supra* caused the board, in its communication pursuant to Article 15(1) RPBA, to question the findings of the opposition division as regards document D4. In this communication, the board also drew the parties' attention to the relevance of document D13, if it was admitted into the proceedings, which, in view of the board's considerations in points 1 to 6 *supra*, is the case.

11. Document D13 is concerned with the production of stable solutions of highly concentrated α-amylase from *Bacillus licheniformis*. The relevance and advantages of these solutions for industrial use are also mentioned in this document (cf. *inter alia*, column 1, lines 1 to 10 and 31 to 42, column 2, lines 24 to 32). The method disclosed for achieving these solutions has several

steps, some of them being optional and/or alternative to other steps (cf. column 2, line 54 to column 3, line 42). Nevertheless, common and critical to all steps and methods is the addition of starch for maintaining the enzyme in solution or, as stated in document D13, for inhibiting enzyme-enzyme agglomeration thereby enhancing the solubility of the alpha-amylase in solution (cf. *inter alia*, column 2, lines 1 to 23 and 33 to 35, column 3, lines 27 to 33). The starting material is a primary filtrate from a *B. licheniformis* culture, i.e. a primary culture filtrate or supernatant (cf. column 6, line 59 to column 7, line 2).

12. In Example II of document D13 (cf. column 8, lines 23 to 53), a culture filtrate is concentrated 7-fold by ultrafiltration and then evaporated to obtain a high concentration of the enzyme. After pH adjustment, the concentrate is divided into four portions and the starch Maltrin-20 is added to each portion in various concentrations. The samples are then stirred moderately for 40 hours, centrifuged and the percentage of enzyme lost in the precipitate calculated. Table II shows that, whereas without Maltrin-20 84% of the enzyme precipitates, only 6.2% of the enzyme precipitates in presence of 8% w/v Maltrin-20, which is regarded in document D13 as "*a drastic decrease in loss of enzyme due to precipitation*" (cf. column 8, lines 49 to 50).

13. In the light of the interpretation of the claims made in point 8 *supra*, the board fails to see any difference between the method disclosed in document D13, in particular the method exemplified in Example II, and the method of claim 1 of the main request. Both

ultrafiltration and evaporation are recovery steps (cf. point 8.1 *supra*). Further, in line with the board's interpretation, the α-amylase is in solution but above its solubility limit, since there is no reference in Example II to the appearance of any enzyme precipitate during the evaporation step or immediately thereafter when Maltrin-20 is added, but only later as a result of stirring and centrifugation (cf. points 8.4 and 8.5 *supra*). The presence of a high amount of precipitate in the solution without Maltrin-20 is an indication that the enzyme is "*in solution but above its solubility limit*" (cf. points 8.2 and 8.3 *supra*). Thus, all features characterizing the method of claim 1 of the main request are anticipated by document D13.

14. The features introduced into claim 1 of auxiliary requests 1 and 2, namely "*without having the protein of interest in precipitated form*" and the protein of interest being "*fully dissolved*" (cf. Sections XII and XIII *supra*), do not overcome the above novelty objection vis-à-vis document D13. Nor is this objection overcome by the feature introduced into claim 1 of auxiliary request 3, namely "*wherein the carbohydrate and/or the polyol is added immediately after a recovery concentration unit step*" (cf. Section XIV *supra*), since both ultrafiltration and evaporation are defined as recovery concentration steps in the patent itself. The combination of these features as present in claim 1 of auxiliary requests 4 and 5 does not render the claimed subject-matter novel over document D13 (cf. point 8.1 *supra*). The contents of this document also anticipate the subject-matter of claim 1 of auxiliary request 6 since the α-amylase is concentrated by ultrafiltration and evaporation steps and Maltrin-20 is added to the

resulting concentrate immediately after these steps (cf. Section XV and point 8.5 *supra*).

15. The feature introduced into claim 1 of auxiliary request 7, namely that in the recovery step the protein of interest is "*concentrated in a continuous mode to form a concentrate*" (cf. Section XVI *supra*), is to be broadly interpreted (cf. point 7 *supra*). In the context of the method disclosed and alternative steps thereof, document D13 refers to a "*continued evaporation*" (cf. column 3, lines 3 to 6 and 36). There is no reason to give another, different meaning to the feature "*continuous mode*" or to read this feature as requiring a particular interpretation not derivable from the claim itself. Thus, document D13 is considered to anticipate the subject-matter of auxiliary request 7.

16. It follows from the above considerations, that the main request and auxiliary requests 1 to 7 do not fulfil the requirements of Article 54 EPC.

Auxiliary requests 8 to 11

17. As stated in Sections V and XXIII *supra*, these auxiliary requests were filed by the respondents in the event that document D13 was admitted into the appeal proceedings. These requests are understood by the board to be a direct reply to the novelty objection raised against the hierarchically higher requests. In view of the relevance of document D13 and the conclusions arrived at above for auxiliary requests 1 to 7, the board considers that, in the present case and for the sake of efficiency, it is more appropriate to assess the requirements of Articles 54 and 56 EPC than to

perform an analysis of whether auxiliary requests 8 to 11 satisfy the formal requirements of the EPC as to patentability (see Articles 123 and 84 EPC).

Article 100(a) EPC; Article 54 EPC

18. The method of claim 1 of auxiliary request 8 has been limited by the deletion of the term "*carbohydrate*" (cf. Section XVII *supra*). In line with the criteria established in the case law (cf. point 7 *supra*), the board considers the term "*polyol*" as embracing "*carbohydrates*", in particular low molecular carbohydrates such as monosaccharides and disaccharides comprised in the composition of Maltrin-100 or used in Example IV of document D13 (cf. column 4, lines 56 to 61 and column 10, lines 13 to 14 of document D13). The results shown in Example IV for these disaccharides (glucose, maltose) are comparable to those reported in the patent when using monopropylene glycol alone and they may only reflect the dependency of the enzyme solubility on various parameters, such as the pH (cf. page 10, paragraph [0090] of the patent and points 8.2 and 8.3 *supra*).

19. The polyol used in the method of claim 1 of auxiliary requests 9 to 11 has been limited to those of a general formula (auxiliary requests 9 and 10) or to seven specific polyols (auxiliary request 11) (cf. Sections XVIII to XX *supra*). None of the products disclosed in document D13 falls within the definition of polyol given in these auxiliary requests. Although both tetra- and pentasaccharides are present in the composition of Maltrin-100 (cf. column 4, lines 59 to 61 of document D13), the latter are given as "*Pentasaccharides, &*

Above" and thus, not limited to the specific ranges defined in auxiliary requests 9 and 10. Nor are the specific polyols of auxiliary request 11 disclosed in document D13.

20. Thus, whereas the subject-matter of auxiliary request 8 is anticipated by document D13, that of auxiliary requests 9 to 11 is not anticipated.

Auxiliary requests 9 to 11
Article 100(a) EPC; Article 56 EPC

21. According to the problem-and-solution approach, when assessing inventive step, it is first necessary to define the closest prior art document. In the decision of the opposition division (cf. page 4, point 14.1 and page 5, point 14.2), document D3 was considered to represent the closest prior art document to the subject-matter of the granted claims. The board, in its communication pursuant to Article 15(1) RPBA, informed the parties of its preliminary opinion that, for the assessment of inventive step, document D13 could be considered as closest prior art, if it was admitted into the proceedings, which is, in view of the board's considerations in points 1 to 6 *supra*, the case.

22. The teachings of document D13 are not limited to the specific starch exemplified (Maltrin) (cf. column 4, lines 21 to 23). Although a starch forming a stable enzyme/substrate complex may be preferred (cf. column 5, lines 6 to 10), document D13 explicitly refers to a general theory of stabilization of enzymes at high concentration and to the properties of the factors required thereto (cf. column 5, line 66 to

column 6, line 30). In Example IV, the results of assays with other factors, such as the low molecular weight maltose and glucose, are reported, although admittedly with no success (cf. column 10, lines 18 to 21 and Table IV of document D13; see also for comparison, paragraph [0090] of the patent and point 18 *supra*).

23. Starting from document D13 taken as the closest prior art, the technical problem to be solved is the provision of an alternative method for achieving solutions of high enzyme (protein) concentration, i.e. reducing enzyme precipitation, by using a factor other than the starch/polyol disclosed in document D13. Although there is no example in the patent, the board considers that, in view of the prior art on file and the parties' arguments, the subject-matter of auxiliary requests 9 to 11 solves the formulated technical problem.

24. A skilled person would be prompted by the disclosure of document D13 to look for other factors to use in the method disclosed for recovering the enzyme/protein of interest. The board is also convinced that, when looking for these factors, a skilled person would consider the disclosure of document D4, since this document is also concerned with bacterial amylase solutions, refers to enzyme recovery and production of solutions of highly concentrated enzyme, and intends to avoid the presence of turbidity (precipitated enzyme) in these solutions (cf. *inter alia*, column 1, lines 34 to 36 and 53 to 59). According to document D4, this is achieved by using polyhydric alcohols, such as propylene glycol, derivatives thereof and glycerol

(cf. *inter alia*, column 3, lines 8 to 15, column 5, lines 43 to 68).

25. The board thus concludes that there is nothing inventive in the selection of these specific products since, as already noted in the board's communication under Article 15(1) RPBA (cf. Section VII *supra*), the addition of glycerol as a stabilizer at the end of purification (recovery) processes for stabilizing and maintaining a purified, homogeneous enzyme in solution (so as to avoid the appearance of turbidity and loss of enzymatic activity) is a standard practice in enzymology. The ability of these products to increase and enhance the solubility of enzymes/proteins is also derivable from the general prior art on file, such as documents D5 (cf. page 124, point 8.4.3) and D11 (cf. page 313, Table 1).

26. Thus, the subject-matter of auxiliary requests 9 to 11 does not fulfil the requirements of Article 56 EPC.

Conclusion

27. As neither the main request nor any of the auxiliary requests 1 to 11 may serve as a basis to maintain the patent, in absence of any further claim request on file, the patent must be revoked.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

A. Wolinski T. J. H. Mennessier