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D E C I S I O N
of 14 June 2006

Case Number: T 0946/02 - 3.3.04

Application Number: 94911856.6

Publication Number: 0691982

IPC: C07K 1/14

Language of the proceedings: EN

Title of invention:
Separation of Proteins

Patentee:
Novozymes A/S

Opponent:
GENENCOR INTERNATIONAL INC.

Headword:
Separation of Proteins/NOVOZYMES

Relevant legal provisions:
EPC Art. 54, 56, 83

Keyword:
"Main request, auxiliary requests II, V, VIII, XI, XIA and XII - Sufficiency of disclosure (no)"
"Auxiliary request XIII - Added subject-matter (no), novelty, inventive step, sufficiency of disclosure (yes)"

Decisions cited:
T 0226/85, T 0019/90

Catchword:
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Case Number: T 0946/02 - 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 14 June 2006

Appellant: (Opponent)
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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 19 August 2002 rejecting the opposition filed against European patent No. 0691982 pursuant to Article 102(2) EPC.

Composition of the Board:

Chairman: U. Kinkeldey
Members: M. Wieser
D. Rogers

Summary of Facts and Submissions

I. The appeal was lodged by the Opponent (Appellant) against the decision of the Opposition Division to reject the opposition against European patent No. 0 691 982 under Article 102(2) EPC. The patent had been granted on the basis of claims 1 to 9. It had been opposed in its entirety under Article 100(a) EPC on the grounds of lack of novelty (Article 54 EPC) and inventive step (Article 56 EPC), under Article 100(b) EPC on the ground of lack of sufficient disclosure (Article 83 EPC).

II. Claim 1 as granted read:

"A method for separating an enzyme from an aqueous solution comprising the enzyme in mixture with other proteins, which method comprises leaching out salts from the solution, followed by adjusting of the pH of the solution to a level around pI of the enzyme, and subsequent recovery of the enzyme on crystalline form."

Dependent claims 2 to 9 referred to preferred embodiments of the method of claim 1.

III. The Board expressed their preliminary opinion in a communication dated 16 December 2005. Oral proceedings were held on 14 June 2006.

IV. The Appellant requested that the decision under appeal be set aside and that the patent be revoked.

The Patentee (Respondent) requested that the appeal be dismissed (main request); or that the decision under

appeal be set aside and the patent maintained upon the basis of auxiliary requests II, V, VIII and XI, filed on 14 May 2003; or auxiliary request XIA, filed at the oral proceedings; or auxiliary request XII, filed on 7 April 2006; or auxiliary request XIII, filed at the oral proceedings.

V. Claim 1 of Respondent's auxiliary requests read as follows:

Auxiliary request II

"A method for separating an enzyme from a culture broth comprising an aqueous solution comprising the enzyme in mixture with other proteins, which method comprises leaching out salts from the solution, followed by adjustment of the pH of the solution to a level around pI of the enzyme, and subsequent recovery of the enzyme from the solution in crystalline form."

Auxiliary request V

"A method for separating an enzyme from a culture broth comprising an aqueous solution comprising the enzyme in mixture with other proteins, which method comprises leaching out salts from the solution until a conductance of 10 mS/cm or less is detectable, followed by adjustment of the pH of the solution to a level around pI of the enzyme, and subsequent recovery of the enzyme from the solution in crystalline form."

Auxiliary request VIII

"A method for separating an enzyme from a culture broth comprising an aqueous solution comprising the enzyme in mixture with other proteins, which method comprises leaching out salts from the solution until a conductance of 5 mS/cm or less is detectable, followed by adjustment of the pH of the solution to a level around pI of the enzyme, and subsequent recovery of the enzyme from the solution in crystalline form."

Auxiliary request XI

"A method for separating an enzyme from a culture broth comprising an aqueous solution comprising the enzyme in mixture with other proteins, which method comprises leaching out salts from the solution until a conductance of 2 mS/cm or less is detectable, followed by adjustment of the pH of the solution to a level around pI of the enzyme, and subsequent recovery of the enzyme from the solution in crystalline form."

Auxiliary request XIA

"A method for separating an enzyme from an aqueous solution comprising the enzyme in mixture with other proteins, which method comprises leaching out salts from the solution, followed by adjustment of the pH of the solution to a level around pI of the enzyme, and subsequent recovery of the enzyme in crystalline form, wherein the enzyme is a lipase."

Auxiliary request XII

"A method for separating an enzyme from a culture broth comprising an aqueous solution comprising the enzyme in mixture with other proteins, which method comprises leaching out salts from the solution until a conductance of 2 mS/cm or less is detectable, followed by adjustment of the pH of the solution to a level around pI of the enzyme, and subsequent recovery of the enzyme from the solution in crystalline form, wherein the enzyme is a lipase."

Auxiliary request XIII

"A method for separating an enzyme from an aqueous solution comprising the enzyme in mixture with other proteins, which method comprises leaching out salts from the solution, followed by adjustment of the pH of the solution to a level around pI of the enzyme, and subsequent recovery of the enzyme in crystalline form, wherein the enzyme is a Humicola lanuginosa lipase or a Rhizomucor miehei lipase."

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

(1) Robert C. Scopes: "Protein Purification-Principles and Practice", 1982, Springer Verlag, Chapters 3 and 9, pages 39 to 65 and 256 to 259

(2) E.L.V. Harris and S. Angal: "Protein Purification Methods-a practical approach", 1989, Oxford University Press, Chapter 3, pages 149 to 155

(3) E.L.V. Harris and S. Angal: "Protein Purification Methods-a practical approach", 1990, Oxford University Press, Chapter 3, pages 45 to 57

(4) A. McPherson: "Preparation and Analysis of Protein Crystals", 1982, John Wiley & Sons, Chapters 1 and 4, pages 6 to 11 and 108 to 123

(5) Eur. J. Biochem., 1990, vol.189, pages 1 to 23

(7) EP-A-0 238 023

(8) EP-A-0 305 216

(9) Experimental data submitted by the Respondent on 23 April 2001 as document (A1)

(10) Experimental data submitted by the Respondent on 15 March 2002 as document (A2)

(20) Experimental data submitted by the Appellant on 23 December 2002 as document (01)

(21) Declaration of Prof. A. McPherson with Annexes I to V, filed by the Appellant on 25 March 2003

(22) Experimental data submitted by the Respondent on 14 May 2003 as document (A4)

(23) Experimental data submitted by the Respondent on 14 May 2003 as document (A5)

(24) Experimental data submitted by the Respondent on 14 May 2003 as document (A6)

(25) Experimental data submitted by the Respondent on 14 May 2003 as document (A7)

(29) Statement by Prof. A. McPherson filed by the Appellant on 11 January 2005.

VII. The submissions made by the Appellant as far as they are relevant to the present decision may be summarised as follows:

The exact conditions required to achieve crystallisation of an enzyme vary from case to case. This applies to crystallization from solutions only containing the desired enzyme and, even more so from solutions containing the desired enzyme in a mixture with other proteins. Therefore, the existence of a method for successfully crystallizing any enzyme from any mixture was not realistic.

The examples of the patent demonstrated that the use of pH adjustment to around the pI at low ionic strength allowed the precipitation of crystals of two types of lipases from exactly defined mixtures. The extra data submitted later by the Respondent added that also some other enzymes could be obtained in crystalline form by a method falling within the scope of claim 1. However, this late filed data contained technical information which was not given in the examples of the patent and which moreover was not said to be of importance for successfully carrying out the claimed method. This technical information, amongst others, concerned the pH and the enzyme concentration of the starting solution. It was noted that the values for these decisive process

parameters varied from example to example, which signalled that they required individual adaptation for each crystallisation process.

It has been shown by the experimental data submitted by the Appellant, that the claimed method did not work in the simple form as described in claim 1 of the main request for the overwhelming majority of proteins. Essential parameters, like starting pH and enzyme concentration, were not discussed or designated as being relevant in the patent. Their importance was highlighted by the Respondent only when Appellant's experiments were criticised as not being a bona-fide attempt to carry out the claimed method.

Crystallization of enzymes was an empirical system dependent on a multitude of parameters. To find a proper crystallization protocol for an enzyme, which moreover was present in a mixture with other proteins, was a complex and multi-iterative process which amounted to undue burden. The specific working examples submitted by the Respondent were of no help for a skilled person trying to perform the claimed method for another enzyme in a different solution. Accordingly the invention was not disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Article 83 EPC).

The additional feature contained in the Respondent's auxiliary requests was not able to provide the skilled person with the information necessary to perform the claimed method without undue burden. Even the invention according to auxiliary request XIII, which was not explicitly restricted to the technical teaching of

original experiments 1 to 4, was not sufficiently disclosed and contravened the requirements of Article 83 EPC.

VIII. The submissions made by the Respondent as far as they are relevant to the present decision may be summarised as follows:

The method according to claim 1 of the main request comprised two working steps, which had to be performed in the defined sequence, namely leaching out of salts followed by an adjustment of the pH. These working steps were known per se. The claimed method did not refer to the crystallisation of enzymes which could not be crystallized by performing these two working steps. The patent as well as the experimental data submitted later by the Respondent had shown that the claimed method could be successfully applied to a broad group of enzymes.

The experimental data submitted by the Appellant with the intention to show that it was not possible to crystallize various enzymes and other proteins by following the method according to the invention, were not representative for tests carried out by a skilled person trying to get the invention to work. First of all some of the examples were concerned with proteins rather than with enzymes, secondly, various parameters chosen by the Appellant, like concentration of the starting solution and pH at the beginning of leaching out of salts, would not have been considered useful by a skilled person willing to succeed.

Appellant's technical expert, Prof. McPherson, was not representative of a notional person skilled in the art of separation of enzymes on an industrial scale, but was rather over-qualified.

The patent contained repeatable examples and it could be shown that the invention could be practised by a skilled person with numerous enzymes when an attempt is made to get the method to work. This might involve routine optimisation of parameters such as starting pH and concentration, but there was no undue burden for a skilled person in doing so. This involved only the application of the skilled person's general knowledge, as disclosed in several prior art documents on file.

Claim 1 of auxiliary request II was restricted to a method for crystallizing an enzyme from a culture broth. This limitation gave an indication of the enzyme concentration and of the composition of the starting solution. The number of variables was therefore restricted and the claim was closely linked to industrial crystallization.

The number of variables was further reduced in claim 1 of auxiliary requests V, VIII and XI, which additionally disclosed the conductance of the solution at the end of the leaching out of salts.

Auxiliary requests XIA and XII were further restricted to the crystallisation of a lipase. Enzymes of this group, besides their functional activity, have in common the feature that they are highly hydrophobic. This common feature could be regarded as an indication for a similar crystallographic behaviour of all lipases.

A method for crystallizing the two specific lipases of claim 1 of auxiliary request XIII was explicitly described and successfully carried out according to examples 1 to 4 of the patent.

Reasons for the decision

1. In the light of the decision taken with regard to the requirements of Article 83 EPC, the Board does not consider it to be necessary to give a reasoned decision with regard to novelty (Article 54 EPC) or inventive step (Article 56 EPC) of the main request and of auxiliary requests II, V, VIII, XI, XIA and XII.

Sufficiency of disclosure - Article 83 EPC

Main Request

2. The patent in suit refers to a method for separating an enzyme from an aqueous solution comprising the enzyme in mixture with other proteins, by recovering it in crystalline form.

The claimed method comprises leaching out of salts from the solution, **followed** by adjustment of the pH of the solution to a level around pI of the enzyme, and **subsequent** recovery of the enzyme in crystalline form.

The patent states on page 2, line 20, that "[t]he use of low conductivity and pH around pI is in itself far from a new method for crystallization of proteins." However, it is stated, that nobody has ever used these

well known working steps in the claimed sequence for purification of enzymes in mixtures with other proteins on an industrial scale.

The Respondent emphasises that it is of great importance for an industrial purification process according to present claim 1, that crystallization of the desired enzyme does not occur spontaneously during leaching out of salts but only after pH adjustment to pI of the enzyme, which can take place in a separate vessel.

The claimed method is said to be applicable to the separation of any enzyme, however preferably it refers to the separation of lipases from any mixture containing them (page 3, lines 6 to 8 of the patent).

The starting solutions referred to in the patent comprise the desired lipase in concentrations as low as 1% (w/w) up to 20% (w/w). In addition the solutions may contain more than 50% (w/w) of other proteins (page 3, lines 9 to 12, and lines 42 to 45).

The ionic strength of the solution is reduced by removal of low molecular impurities (leaching out of salts) to a conductance of 10 mS/cm or less. Thereafter the pH of the solution is adjusted to a value in the range of +/- 3 around the pI of the lipase (page 3, lines 33 to 37).

Examples 1 to 4 describe the crystallization of two lipases from a culture broth, namely *Humicola lanuginosa* lipase from a broth obtained as described in

document (8) and *Rhizomucor miehei* lipase obtained as described in document (7).

The following parameters are indicated in said examples:

- cut-off value of the ultra/diafiltration membrane,
- conductance of the solution after ultra/diafiltration,
- lipase concentration of the solution after ultra/diafiltration,
- total dry substance of the solution after ultra/diafiltration,
- pH value after adjustment,
- crystallization time and temperature,
- yield and purity of the crystallized lipase.

3. During the opposition procedure the Respondent submitted documents (9) and (10), which report the results of further examples. Additional experimental data was submitted during appeal procedure (documents (22) to (25)).

Documents (9) and (10) describe the successful crystallisation of two different α-amylases, a cellulase and a pectin lyase. In addition to the information given in the examples of the patent specification (see point (2) above), documents (9) and (10) indicate the starting pH of the culture broth, i.e. before ultra/diafiltration. This pH value differs from example to example (8,5 and 11 for the two α-amylases, obtained from different Bacillus strains, 8 for the cellulase and 7 for the pectin lyase).

Document (22) describes the crystallisation of an endopeptidase, a protease and a xylanase. The starting pH of the solution (before ultra/diafiltration) is indicated for all three examples and varies between 5,6 (protease) and 8 (endopeptidase).

Document (23), in which the same protease is used as in document (22), highlights the importance of pH adjustment after ultra/diafiltration. If no such adjustment is performed, no crystallisation occurs, if the pH of the solution is adjusted to a value of 0,5 units below the pI of the protease, the enzyme is separated in crystalline form. The starting pH before ultra/diafiltration is indicated.

Document (24) refers to the crystallisation of an α-amylase from a *Bacillus sp.* strain. The same enzyme was used in example 2 of document (9). It is stated in document (24) that the pH of the solution after ultra- and before diafiltration is 10,5 (according to document (9) the pH of the culture broth before ultra/ diafiltration is 11). It is further shown that a pH adjustment to 7 before diafiltration results in the formation of a heavy precipitate almost entirely blocking the membrane, while the enzyme solution remains clear during diafiltration without this pH adjustment. Adjustment of pH to a value of 1,2 above the pI of the α-amylase after diafiltration results in crystallisation of the enzyme. No crystallisation is achieved without pH adjustment after diafiltration.

The experimental data of document (25) are identical to the data presented in documents (9),(10) and (22).

4. The Appellant has filed experimental data in documents (20),(21) and (29).

Document (20) is the report of three unsuccessful experiments to crystallise different enzymes according to the following method:

A defined culture broth containing the desired enzyme is clarified by rotary vacuum drum filtration. The filtrate is ultra/diafiltered to low ionic strength (conductivity values 2,74 mS/cm, 0,934 mS/cm and 1,10 mS/cm). Thereafter the pH of the solution is adjusted to the respective pI of the enzyme.

While the solution after pH adjustment in example 1 (*Bacillus amyloliquefaciens* protease) remains clear at three different temperatures with and without mixing, the samples in examples 2 and 3 (*Trichoderma reesei* xylanase and *Micrococcus luteus* catalase) show precipitate formation. No crystals are observed.

In Annex (IV) of document (21), the technical expert of the Appellant, Prof. McPherson, reports the results of crystallisation investigations. Fourteen commercially available enzymes and other proteins are dissolved in buffer pH 6,5 to concentrations of 30 mg/ml (3% (w/v)) and three culture broths, each containing one of the enzymes described in the examples of document (20), are clarified by centrifugation and are additionally dialysed versus distilled water at 4°C over a total period of 48 hours. After this extensive dialysis to zero ionic strength and before any adjustment of pH, the seventeen examples are examined visually and under a light microscope with polarization filters (table II,

document (21). While seven samples remain completely clear (among them the three culture broths), the others have developed some insoluble material. In four cases this material consists of microcrystals (proteinase, beef catalase, elastase and lipase). The remaining six samples contain precipitates of some sort. The solid material is thereafter removed from the samples not containing crystals and the pH of the solutions is adjusted to the pI of the enzyme (protein) contained therein. Then the samples are placed at 4°C for 48 hours. The four samples that have produced crystals only upon dialysis remained unchanged, all other samples either remain clear or produce precipitates of some sort (table III of document (21)). One sample, containing Concanavlin A, after repeated concentration, clarification and pH adjustment produces crystalline material. Thus, enzymes (proteins) are obtained in crystalline form in four out of seventeen initial samples upon dialysis against water alone, and in one more sample upon dialyses, concentration and repeated pH adjustment. Twelve out of seventeen samples remain either clear or produce some precipitate.

In summary, crystallisation of the desired enzyme (protein) is not achieved **subsequent** to leaching out of salts **followed** by pH adjustment in one out of seventeen trials.

Document (29) contains additional experiments investigating the influence of dialysis on the pH and conductance of enzyme containing solutions.

5. The Respondent commented on the Appellant's unsuccessful experiments presented in document (20) by

criticizing the fact that no starting pH of the solutions was indicated and that the enzyme concentrations used were either too high or too low (letter of 14 May 2003; points (1.6.9) to (1.6.14)).

The experiments of document (21) were commented on in point (2.4) of the same letter. The main points of criticism were: several of the investigated proteins were not enzymes; no indication of the starting pH before dialysis; unclear starting concentration and purity of the used samples; too high intensity of dialyses (to zero ionic strength).
The notional skilled person as defined in the case law of the Boards of Appeal, in contrast to Prof. McPherson, who was considered to be over-qualified, would have reacted differently when being confronted with initial failure. He would have succeeded in performing the experiments in a successful way by relying on his/her general knowledge.

The Respondent further argues that the patent contains repeatable examples and that even the Appellant's technical expert was able to practise the invention with some enzyme-containing solutions. Further, the routine optimisation of parameters like starting pH and enzyme concentration does not amount to undue burden, but requires nothing more than a skilled person's general knowledge. Useful information for the definition of a crystallisation protocol for a defined enzyme can be found in documents (1) to (5) which are considered to give a picture of the skilled person's general knowledge at the filing date of the present patent. Document (5) lists relevant crystallisation parameters and provides methods, arrays and matrices

that enable the skilled reader to define in a straightforward way the key parameters for successfully crystallizing an enzyme from a solution.

6. Article 83 EPC stipulates that **a patent** must disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

The disclosure must be reproducible without undue burden.

Even though the case law of the Boards of Appeal accepts that the skilled person may be subject to a reasonable amount of trial and error when it comes to sufficiency of disclosure, e.g. in an unexplored field or where there are many technical difficulties, the skilled person has to have at his disposal, either in the specification or on the basis of common general knowledge, adequate information leading necessarily and directly towards success through the evaluation of initial failures (cf decisions T 226/85, OJ EPO 1988, 336, point (8)).

7. In the present case the subject-matter of claim 1 is a method for separating an enzyme in crystalline form from a solution comprising said enzyme in mixture with other proteins. The scope of the claim encompasses separation of any enzyme from any solution containing any other protein or proteins. The claimed method is defined by two working steps, leaching out of salts and adjustment of the pH to a level around pI of the enzyme, which working steps have to be performed in this sequence. No other process parameters defining the

claimed method and thus restricting the scope of the claim are indicated. The term "leaching out of salts" has to be interpreted in the meaning of the description where it is said that the conductance of the solution is reduced to less than 10 mS/cm (page 3, lines 33 to 35). The term "adjustment of the pH to a level around pI of the enzyme" has to be interpreted as meaning an adjustment to a value of +/- 3 around the pH of the enzyme (page 3, lines 36 to 39).

8. The patent shows that the claimed method, under specific circumstances, may allow the recovery of an enzyme in crystalline form (see examples 1 to 4).

The question that arises under Article 83 EPC is, whether or not these successful examples, which were carried out under defined conditions, namely by using defined culture broths containing a specific enzyme in mixture with specific other proteins under defined process parameters, like, among others, pH and enzyme concentration of the starting solution, are of any help for a skilled worker trying to perform the claimed method with any other protein containing solution comprising a different enzyme.

9. Document (5) reads on page 8 (passage bridging left and right column) as follows:

"Crystallisation of a novel protein using any of the precipitation methods is unpredictable as a rule. Every macromolecule is unique in its physical and chemical properties because any amino acid or nucleotide sequence produces a unique three-dimensional structure having distinctive surface characteristics. Thus,

lessons learned by investigation of one protein are only marginally applicable to others. This is compounded by the behaviour of macromolecules which is complex owing to the variety of molecular masses and shapes, aggregate states, and polyvalent surface features that change with pH and temperature, and to their dynamic properties."

This statement in document (5), whose author is Prof. McPherson, Appellant's technical expert, sharply contradicts Respondent's argument, that optimisation of process parameters for crystallisation of enzymes from impure solution requires nothing more than general knowledge of a skilled person.

10. Enzymes, belonging to the chemical group of proteins, due to their complex structure, do not behave in a uniform way.

In this context the Board holds, that Respondent's argument that Appellant's experiments of document (21) are not representative because they refer partly to proteins rather than enzymes must fail. Firstly, the majority of the examples (thirteen out of seventeen) are concerned with enzymes, secondly, all proteins used in the examples have a well-defined three-dimensional structure, as enzymes do.

11. Crystallisation of proteins is an empirical process dependent on many parameters, which mutually interact. This opinion, which is expressed in document (5) (see point (9) above), is repeated by Prof. McPherson, an acknowledged expert in the field of protein crystallisation, in a declaration filed as document (21)

and a statement filed as document (29) in the present case. It is moreover supported by Respondent's own late filed experiments, which disclose at least some of the parameters not indicated in the examples of the patent, like the starting pH. These parameters take a different value in each of the late filed experiments, which can be considered as being a sign of the unpredictability of protein-crystallisation-systems.

12. The patent in suit does not disclose any of the parameters, which the Respondent, when criticizing Appellant's experiments, considers as being of importance for successfully carrying out the claimed method. Moreover, the patent besides from being silent with regard to these parameters, does not contain information that would prompt the skilled worker facing initial failure to focus on these parameters and to try to optimise the crystallisation protocol for the desired enzyme by adapting them. Respondent's argument that such optimisation is a routine task which requires nothing more than general knowledge, is an allegation, which is not substantiated by verifiable facts and contradicted by the state of the art, as represented by the disclosure in document (5).

13. In the light of the evidence on file, the Board comes to the conclusion that in the present case the disclosure of specific successful working examples in the patent are of no help for a skilled person trying to exercise the invention in a different set-up, namely with a different enzyme in a different solution. For each and every different enzyme the skilled person has to start from scratch and has to find the suitable parameter values for the relevant crystallisation

protocol, which is not a trivial routine task, but a highly iterative process requiring skill and inventive activity and thus amounts to undue burden.

14. Therefore, contrary to the requirements of Article 83 EPC, the patent does not disclose the invention according to Respondent's main request in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

Auxiliary Request II

15. Claim 1 is distinguished from claim 1 of the main request in that it refers to the separation of an enzyme **from a culture broth comprising an aqueous solution.**

The Respondent argues that this additional feature restricts the scope of the claim in so far as the enzyme concentration and the nature and concentration of other proteins which may be present are more precisely defined, which reduces the number of variables of the claimed method. Moreover, the claim is more closely linked to industrial crystallization.

16. In document (20) and in Annex IV of document (21), the Appellant has provided the results of unsuccessful trials to crystallize enzymes from culture broths.

Respondent's argument (point (15), second paragraph, above) may be true for a culture broth with a known composition. However, it does not seem to be able to overcome the deficiencies under Article 83 EPC as

discussed with regard to the main request in points (2) to (13) above.

Due to the fact that many organisms produce very high amounts of a specific enzyme or other protein, some of the parameters most crucial for the claimed method, like staring pH and concentration, may vary widely between different culture broths.

17. Therefore, the Board finds that the skilled person trying to carry out the invention according to auxiliary request II, is faced with the same difficult and complex task which had to be solved with regard to the main request, namely the development of a crystallisation protocol for a specific system, which requires skill and inventive activity and thus amounts to undue burden.

Therefore, auxiliary request II does not meet the requirements of Article 83 EPC.

Auxiliary Requests V, VII and XI

18. Claim 1 of these requests additionally defines the final conductance of the solution after leaching out of salts. The conductance is less than 10 mS/cm in auxiliary request V, less than 5 mS/cm in auxiliary request VIII and less than 2 mS/cm in auxiliary request XI.

The Respondent argues that the introduction of this feature further reduces the number of variables of the claimed method.

19. The term "less than 10 mS/cm" ("less than 5 ms/cm"; "less than 2 mS/cm") includes the value zero.

According to Annex IV of document (21) the seventeen tested samples were dialysed versus distilled water at 4°C over a total period of 48 hours to zero ionic strength (see point (4) above). In none of these experiments was the Appellant able to successfully recover the desired enzyme subsequent to dialyses and pH adjustment, as required according to claim 1 of auxiliary requests V, VIII and XI.

Consequently, these requests, for the same reasons as for the main request and auxiliary request II, do not meet the requirements of Article 83 EPC.

Auxiliary requests XIA and XII

20. Claim 1 of auxiliary request XIA is distinguished from claim 1 of the main request in that it defines the enzyme as being a lipase.

The same feature distinguishes claim 1 of auxiliary request XII from claim 1 of auxiliary request XI.

21. The claims have been restricted to a specific class of enzymes. The Respondent argues that all members of this group not only perform the same biological activity, namely catalysing the hydrolyses of ester bonds in water-insoluble, lipid substrates, they also share a common structural feature as they are all highly hydrophobic. This could be an indication of a common "crystallisation-behaviour". The patent contains four examples wherein the claimed method is successfully

used for the recovery of two lipases in crystalline form. The use of this method for crystallizing another lipase does not amount to undue burden.

22. The group of lipases contains a huge number of different enzymes all sharing the same biological activity. Although it is correct that these enzymes are all hydrophobic, otherwise they could not perform this activity, the Respondent's conclusion that this may be an indication of a common "crystallisation-behaviour" of all lipases is a mere assumption not substantiated by verifiable facts.

The Appellant attempted to crystallize a commercially available lipase in one of the experiments disclosed in Annex IV of document (21). The solution containing the lipase developed microcrystals after dialyses against water alone and did not change after adjustment of the pH to the pI of the lipase (see tables II and III of document (21).The skilled person when trying to generalize the examples contained in the patent in suit in order to crystallize other enzymes belonging to the group of lipases is confronted with the same tasks and problems as in the case for enzymes in general, which thus amounts to undue burden as already identified and discussed by the Board when deciding on the preceding auxiliary requests.

23. Therefore, the invention according to Respondent's auxiliary requests XIA and XII does also not meet the requirements of Article 83 EPC.

Auxiliary Request XIII

Clarity and Amendments - Articles 84, 123(2) and 123(3) EPC

24. Claim 1 is based on claims 1 and 8 as originally filed. Claims 2 to 6 correspond to claims 2 to 5 and 7 as originally filed. The claims are clear and supported by the description.

The formal requirements of Articles 84, 123(2) and 123(3) EPC are met.

Novelty and inventive step - Articles 54 and 56 EPC

25. None of the documents on file refers to or suggests a method for separating a lipase from Humicola lanuginosa or from Rhizomucor miehei in crystalline form from a solution containing it in a mixture with other proteins.

Considering that "crystallisation of a novel protein using any of the precipitation methods is unpredictable", that "every macromolecule is unique" and that "lessons learned by investigating of one protein are only marginally applicable to others" (document (5), page 8), documents referring to crystallisation of proteins in general without addressing the two specific lipases of claim 1, like documents (1) to (5), are not regarded as being relevant prior art for the assessment of inventive step.

No objection due to lack of novelty or inventive step of the claims of auxiliary request XIII has been raised by the Appellant.

Accordingly the requirements of Articles 54 and 56 EPC are met.

Sufficiency of disclosure - Article 83 EPC

26. The Appellant argues that the claims, although referring to the separation of two specific lipases, are not restricted to the subject-matter of examples 1 to 4 of the patent. Claim 1 does not define relevant parameters, like enzyme concentration and pH of the starting solution. Therefore, a skilled person when trying to crystallize the defined enzymes from a solution or culture broth different from the ones used in the examples of the patent is confronted with undue burden. The patent does not disclose this embodiment of the invention, falling within the scope of the claims of auxiliary request XIII, in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

27. According to established case law of the Boards of Appeal an objection based on lack of sufficient disclosure presupposes that there are serious doubts, substantiated by verifiable facts. The mere fact that a claim is broad is not in itself a ground for considering the patent as not complying with the requirement of sufficient disclosure under Article 83 EPC (cf decision T 19/90, OJ EPO 1990, 476, point (3.3) of the reasons).

28. Contrary to the situation in case of the invention according to the preceding auxiliary requests, no such verifiable facts leading to serious doubts are

identified by the Board in the case of the invention according to auxiliary request XIII.

Therefore, the Board finds that the requirements of Article 83 EPC are met.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to maintain the patent upon the basis of claims 1 to 6 of auxiliary request XIII filed during oral proceedings and a description yet to be adapted thereto.

Registrar: Chair:

A. Wolinski U. Kinkeldey