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Datasheet for the decision of 28 August 2025

Case Number: T 2001/23 - 3.3.04

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Title of invention:
Recovery process

Patent Proprietor:
Novozymes A/S

Opponent:
BASF SE

Headword:
Protease recovery/NOVOZYMES

Relevant legal provisions:
EPC Art. 56, 123(2)

Keyword:
Inventive step - main request, auxiliary requests 1 to 3 (no)
Amendments - added subject-matter - auxiliary requests 4 and 5 (yes)

Decisions cited:
G 0002/10, G 0001/24, T 1018/02, T 1473/19, T 2140/22

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Case Number: T 2001/23 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 28 August 2025

Appellant I: (Patent Proprietor)	Novozymes A/S Krogshoejvej 36 2880 Bagsvaerd (DK)
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Appellant II: (Opponent)	BASF SE Carl-Bosch-Straße 38 67056 Ludwigshafen (DE)
Representative:	BASF IP Association BASF SE GBI - Z078 67056 Ludwigshafen (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 18 October 2023 concerning maintenance of the European Patent No. 3 606 936 in amended form**

Composition of the Board:

Chair	M. Blasi
Members:	B. Rutz
	S. Albrecht

Summary of Facts and Submissions

I. The appeals by the patent proprietor (appellant I) and the opponent (appellant II) lie from the opposition division's decision that European patent No. 3 606 936 ("the patent"), which was granted on European patent application No. 18 715 649.2, published under the PCT as international application WO 2018/185048 ("the application as filed"), as amended in the form of auxiliary request 4 fulfilled the requirements of the EPC.

II. This decision will refer to the appellants by their respective roles in the opposition proceedings, i.e. patent proprietor and opponent.

III. The patent had been opposed on the grounds of Article 100(a) EPC, in relation to novelty (Article 54 EPC) and inventive step (Article 56 EPC), and of Article 100(b) EPC.

IV. The opposition division decided that the main request, the claims of which had been filed on 28 July 2023, fulfilled the requirements of Rule 80 and Articles 123(2), 123(3), 83 and 54 EPC, but that the subject-matter of its claim 1 lacked an inventive step (Article 56 EPC). The conclusion on Article 56 EPC applied also to claim 1 of auxiliary requests 1 to 3, likewise filed on 28 July 2023. Moreover, auxiliary request 2 related to subject-matter extending beyond the content of the application as filed (Article 123(2) EPC).

V. In its statement setting out the grounds of appeal, the patent proprietor indicated that it maintained the main

request and auxiliary requests 1 and 3 to 5 underlying the decision under appeal. With the same statement, the patent proprietor filed a set of claims of a new auxiliary request 2 to replace auxiliary request 2 dealt with in the decision under appeal, and filed, as annexes A and B, its submissions from the opposition proceedings, dated 2 December 2022 and 28 July 2023 respectively.

VI. The opponent filed documents D18 to D20 with its statement setting out the grounds of appeal.

VII. Both appellants filed a reply to the other party's appeal.

VIII. In view of the parties' requests, the board summoned the parties to oral proceedings, to be held at the premises of the boards, and informed them of its preliminary opinion in a communication under Article 15(1) RPBA.

IX. Subsequently, the board changed the format of the oral proceedings to a videoconference, as requested by the patent proprietor and consented to by the opponent.

X. Claim 1 of the main request reads as follows:

"1. A method for recovering one or more enzyme from a fermentation broth, where the one or more enzyme is present in precipitated form in the fermentation broth, the method comprising the steps of:

a) a first separation step separating the fermentation broth in a first phase and a second phase, wherein the first phase comprises supernatant, the one or more enzyme in soluble form and optionally cells and cell debris, and the

second phase comprises the one or more enzyme in precipitated form, cells and cell debris; and
b) a solubilization step where the one or more enzyme in precipitated form in the second phase is solubilized,
wherein the solubilization step comprises:
i. Diluting the second phase 100-2000% (w/w) in water or an aqueous medium;
ii. adding a divalent salt; and
iii. adjusting the pH to a pH value below 6.0

wherein the one or more enzyme is selected among proteases having at least 80% sequence identity, such as at least 85% sequence identity, such as at least 95% sequence identity, such as at least 96% sequence identity, such as at least 97% sequence identity, such as at least 98% sequence identity, such as at least 99% sequence identity to one of SEQ ID NO: 1 - 6."

Claim 1 of auxiliary request 1 differs from claim 1 of the main request in that step ii. reads:
"ii. adding a divalent salt, wherein the divalent salt is selected among Calcium, Magnesium, Ferrous and Zinc salts comprising an anion selected among phosphates, sulphate, nitrate, acetate and chloride and is added in an amount of 0.01-5% (w/w) based on the diluted second phase;".

Claim 1 of auxiliary request 2 differs from claim 1 of auxiliary request 1 in that the following wording has been inserted immediately after step iii.:
"wherein before the first separation step, the fermentation broth is subjected to dilution as a pretreatment step,".

Claim 1 of auxiliary request 3 differs from claim 1 of the main request in that the term "precipitated" has been replaced on each occurrence by the term "crystalline".

Claim 1 of auxiliary request 4 differs from claim 1 of the main request in that the following wording has been inserted immediately after step iii.:
"wherein the solubilization step takes place in the absence of the liquid part of the fermentation broth,".

Claim 1 of auxiliary request 5 includes in combination the amendments made to claim 1 of each of auxiliary requests 3 and 4.

XI. Oral proceedings took place on 28 August 2025 in the presence of both parties. At the end of the oral proceedings, the Chair announced the board's decision.

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

D2 DE 199 56 239 A1
D3 WO 93/13125 A1
D4 EP 2 464 446 B1
D5 US 6,316,240 B1
D6 WO 2008/110498 A1
D10 J. A. Grootegoed et al., "*Separation and Partial Purification of Extracellular Amylase and Protease from Bacillus caldolyticus*", Arch. Mikrobiol. 90, 1973, 223-232

XIII. The patent proprietor's submissions relevant to this decision are summarised as follows:

Main request - claim 1
Inventive step (Article 56 EPC)
Document D2 as starting point

D2 was not suitable as the closest prior art. The starting point for the skilled person had to be a fermentation system that, during recovery following fermentation, contained solid enzyme intermixed with enzyme adhered to the biomass. Enzyme in this form was not disclosed in D2, either explicitly or implicitly. It was entirely consistent with the teaching of D2 that the solids that were washed were cell debris (i.e. biomass) which had enzyme retained thereon, which led to solubilised enzymes being recovered when subjected to washing, but not to the solubilisation of enzyme in precipitated form as required by the claim. The primary consideration when selecting the closest prior art was that it lay in the same technical field and was directed to the same technical problem. Beyond that, consideration was then given to how many features it had in common with the invention. In line with the problem-solution approach, the closest prior art had to be directed to a similar purpose or effect as the present invention. D2 was not directed to methods of recovering precipitated enzymes, but rather methods of improving efficiency of solubilisation of enzymes adhered to biomass.

Even if D2 was taken as the closest prior art, the claimed invention was not obvious. The differences between claim 1 and D2 were:

- the pH of the resolubilising solution (pH of below 6.0 vs. optionally pH of 7.5 or slightly higher); and
- this pH was adjusted during the course of a solubilising step comprising diluting at 100-2000% w/w

The problem underlying the invention starting from D2 was the provision of a more resource-efficient process for re-solubilisation of precipitated enzyme.

A combination of D2 and D6 would not have led the skilled person to consider adjusting the pH after separation. D2 only disclosed the concept of washing the biomass with the ultrafiltrate concentrate of the fermentation liquor. There was no disclosure in D2 of intentionally diluting the biomass with anything other than the ultrafiltrate of the solution which was separated from the biomass. Moreover, D2 also clearly taught against using high volumes during this washing step, which ran contrary to the requirement of claim 1 to dilute the biomass 100-2000% w/w.

D6 would not have led the skilled person to modify the teaching of D2 to arrive at the method of claim 1 of the main request. D6 only taught direct modification of the fermentation broth, *inter alia* by pH adjustment. D6 would not have led the skilled person to carry out the pH adjustment during a dilution step after the biomass had been separated.

Auxiliary request 1 - claim 1
Inventive step (Article 56 EPC)

The claim contained a specific list of divalent salts that were used in the solubilisation step. It met the requirements of Article 56 EPC for at least the same reasons as the main request.

Auxiliary request 2 - claim 1
Inventive step (Article 56 EPC)

The claim met the requirements of Article 56 EPC for at least the same reasons as the main request.

Auxiliary request 3 - claim 1
Inventive step (Article 56 EPC)
Document D4 as starting point

D4 related to maintaining an enzyme in precipitated form in order to prevent degradation. The most important test for selecting the closest prior art was that the document was directed to the same technical problem. This was not the case for D4, in which precipitation of an enzyme was clearly the purpose. This was not the purpose of the claimed method. In attempting to improve solubilisation of a precipitated enzyme, it was completely unreasonable to argue that the skilled person would turn to a document that related to purposefully carrying out the opposite process. Moreover, D4 did not disclose proteases, but other enzymes. It referred to crystals only in a longer list of higher molecular weight forms.

If D4 was nevertheless chosen as closest prior art, it was difficult if not impossible to formulate a meaningful problem starting from this document without including a pointer to the solution.

Auxiliary requests 4 and 5 - claim 1
Claim interpretation

The method of claim 1 required a first separation in which the fermentation broth was separated into a first

phase and a second phase. The first phase comprised supernatant, one or more enzymes in soluble form and optionally cells and cell debris. The second phase comprised the one or more enzyme in precipitated form, cells and cell debris.

The common meaning of the word "*supernatant*" was the liquid resulting from the separation of liquids and solids. Once the two phases or parts had been established, the method of claim 1 required that the solid phase (second phase), was treated to solubilise precipitated enzyme. This solubilisation step was carried out "*in the absence of the liquid part of the fermentation broth*", i.e. the solubilisation step had to be carried out in the absence of the first phase or supernatant from which the second phase was separated in the first step.

Amendments (Article 123(2) EPC)

The phrase "*at least part*" (see page 3, lines 29 to 31 of the application as filed) encompassed two alternatives: either (i) some or (ii) all of the liquid part of the fermentation broth should be absent during the solubilisation step.

The application as filed comprised a number of pointers towards performing the solubilisation step in the absence of all of the liquid part of the fermentation broth, wherein in the context of claim 1, the liquid part of the fermentation broth was the first phase. Accordingly, of the two alternatives, the skilled person would understand that the absence of all the liquid part was a preferred embodiment (see e.g. examples in which the solids and liquids are separated in the first step, and the second step is carried out

on the solid fraction only). By removing the liquid part of the fermentation broth, disadvantageous components were also removed, thereby improving solubilisation of precipitated enzyme and leading to the claimed advantage. A method wherein the solubilisation step was performed on the second phase, in the absence of the first phase, was therefore clearly and unambiguously derivable from the application as filed.

XIV. The opponent's submissions relevant to this decision are summarised as follows:

Main request - claim 1
Inventive step (Article 56 EPC)
Document D2 as starting point

D2 could be taken as the closest prior art for assessing inventive step because it lay in the same technical field and had the same purpose, with both relating to recovery processes that enabled the extraction of enzymes, specifically proteases, from a fermentation broth or more specifically from the sludge of the fermentation broth.

The term enzyme precipitate was not further defined in the patent in suit and the different forms of insoluble enzyme were used inconsistently and interchangeably. In D2, all insoluble solid substances were separated in the first separation step and were referred to as "*biomass*", i.e. everything contained in the sludge phase. The filtrate of the ultrafiltration step, which was used in D2 for the washing step, mainly contained water, small solutes that could pass through the ultrafiltration membrane and only trace amounts of enzyme.

The claim differed from D2 in that the pH was adjusted to a pH value below 6.0 instead of the pH adjustment to 7.5 in D2. No beneficial effect had been shown for this alternative solubilisation condition. Thus, the problem to be solved could be considered to be the provision of an alternative method for solubilising proteases remaining in the sludge phase after the first separation step.

D6 suggested using an acidic pH in combination with divalent salts for solubilising protease precipitate. The solubilisation method of D6 was an obvious alternative for the skilled person.

Auxiliary requests 1 and 2 - claim 1
Inventive step (Article 56 EPC)

The claim did not contain any additional features for which a technical effect had been shown and which could confer inventive merit.

Auxiliary request 3 - claim 1
Inventive step (Article 56 EPC)
Document D4 as starting point

D4 disclosed a method that separated a fermentation broth comprising precipitated protein of interest into a first and a second phase, wherein the second phase - comprising the precipitated protein of interest - was subjected to conditions that solubilised the protein of interest contained therein.

The difference of the claim compared to D4 was the use of the specific solubilisation conditions for a protease. No technical effect was shown by this

difference. The solubilisation conditions were all disclosed in D6. For solubilisation of the precipitated protein, D4 itself already suggested adjusting the salt concentration, the precipitant concentration (i.e., dilution), and the pH value (see column 3, paragraph [0017], and claim 10). Hence, the subject-matter was not inventive in view of D4 in combination with D6.

Auxiliary requests 4 and 5 - claim 1
Claim interpretation and amendments
(Article 123(2) EPC)

The passage of page 3, lines 29 to 31 was amended upon incorporation into the claim. The term "*at least part*" and the specification that the liquid part of the fermentation broth should contain "*the majority of the disadvantageous components*" were omitted. The "*whole part*" including the upper end of the range was not disclosed in the application as filed. Firstly, the liquid part of the fermentation broth did not necessarily correlate with the first phase. Secondly, the majority of disadvantageous components were not by nature always in the first phase. Removal of the liquid part of the fermentation broth did not correlate with removal of the majority of compounds detrimental to solubilisation. Thus, the feature "*containing the majority of the disadvantageous components*" was not inherent to the liquid part of the fermentation broth. In the examples, it was not described that the first phase contained the majority of the disadvantageous components and that therefore the solubilisation was performed in the absence of the whole liquid phase. Moreover, in examples 1 and 2, a specific type of centrifuge was used which generated a "*slurry*", i.e. a second phase still comprising a large quantity of liquid. In example 3, too, liquid still remained in the

second phase as drum filtration did not generate a dry filter cake.

Thus, the examples did not contain a pointer to a method comprising solubilisation in the absence of the ("*whole*") liquid part of the fermentation broth either.

The claimed subject-matter thus constituted an unallowable amendment. The same objections applied to claim 1 of auxiliary request 5.

XV. The patent proprietor requested:

- that the decision under appeal be set aside and the patent be maintained in amended form on the basis of the set of claims of the main request underlying the decision under appeal, or alternatively, on the basis of any of the sets of claims of
  - auxiliary request 1 underlying the decision under appeal
  - auxiliary request 2 filed with the statement of grounds of appeal, or
  - auxiliary request 3 underlying the decision under appeal
- further alternatively, that the patent be maintained in the form of auxiliary request 4, i.e. the form held allowable by the opposition division, implying the request that the appellant-opponent's appeal be dismissed
- further alternatively, that the patent be maintained in amended form on the basis of the set of claims of auxiliary request 5 filed during the opposition proceedings

The patent proprietor further requested that documents D18 to D20 not be admitted into the appeal proceedings.

The opponent requested that the decision under appeal be set aside and the patent be revoked in its entirety. The opponent further requested that auxiliary requests 2 and 5 not be admitted into the appeal proceedings.

Reasons for the Decision

Admission of documents D18 to D20 (Article 12(4) and (6) RPBA)

1. The documents, all filed by the opponent with its statement of grounds of appeal, were not relevant to the present decision and the parties did not rely on them during the oral proceedings either. The board therefore did not decide to admit them or to consider them with regard to substance.

Main request - claim 1
Inventive step (Article 56 EPC)
Document D2 as the closest prior art

2. The opposition division in its decision assessed inventive step starting from D2 as the closest prior art. The patent proprietor objected to this, arguing that the closest prior art had to be directed to a similar purpose or effect as the claimed invention. D2 was not directed to methods of recovering precipitated enzymes, but rather to methods of improving efficiency of solubilisation of enzymes adhered to biomass. D2 was therefore not an appropriate starting point for assessing inventive step using the problem-solution approach.

3. The board finds that, in principle and in line with the wording of Article 56 EPC, any prior art can be chosen as a starting point for assessing inventive step. If the subject-matter of a claim is found to lack an

inventive step starting from a particular disclosure, it is not possible to establish inventive step by proposing that the assessment be started from different prior art. Article 56 EPC requires the subject-matter to be obvious over "*the state of the art*" (see Case Law of the Boards of Appeal of the EPO, 11th edition 2025, I.D.3.1 to 3.3).

4. The board furthermore does not agree with the patent proprietor that D2 did not disclose an "*enzyme in precipitated form*" as required by the claim. To arrive at this conclusion the board, in the following, first interprets the wording of claim 1 and then addresses the disclosure of D2.

5. The method of claim 1 separates a fermentation broth into a first and second phase. An "*enzyme present in precipitated form in the fermentation broth*", i.e. already in precipitated form before the separation, thereby arrives in the second phase together with cell and cell debris. This means that the enzyme is present in the fermentation broth in a form which allows it to be separated, e.g. when the broth is centrifuged or filtered.

6. The opponent considered that although the claim recited "*enzyme in precipitated form*" in the second phase the patent did not distinguish between the different insoluble enzyme forms. Therefore, it had to be assumed that following the claimed method, all different kinds of insoluble enzyme were solubilised and not only the "precipitated" form.

7. The patent proprietor argued that by consulting the description (in line with decision G 1/24, Order), the skilled person would be aware that "*enzyme in*

precipitated form" was different from enzyme bound to cell mass as apparent from the patent in paragraph [0018], which stated that "*part of the fermentation product may precipitate forming crystals or amorphous precipitates in the fermentation broth*" and paragraph [0055], which listed "*crystals and/or precipitate and/or desired product bound to cell mass/insolubles*" as alternatives.

8. The board has doubts about the patent proprietor's interpretation of the feature "*enzyme in precipitated form*" and in particular about whether this feature excludes enzyme bound to cell mass/insolubles. The board, however, does not consider it necessary to come to a conclusion on this issue because adopting the patent proprietor's interpretation does not affect the outcome of this decision. Hence, in the following, the board adopts the patent proprietor's interpretation of the feature, i.e. "enzyme in precipitated form" does not include enzyme bound to cell mass/insolubles.

9. D2 discloses a method for the recovery of protease, e.g. Savinase® (column 2, lines 36 to 41), from a fermentation broth comprising $CaCl_2$ and having a pH of 7.5 (see example), wherein the broth is separated into two phases by centrifugation (see e.g. column 2, lines 46 to 59). Residual enzyme present in the mostly solid phase is extracted or dissolved out ("*herausgelöst*") by washing the solid phase with the filtrate (see column 1, lines 60 to 64). It was undisputed between the parties that Savinase® was an enzyme falling under the definition of claim 1.

10. Referring to the example of D2, the patent proprietor initially argued that the dilution achieved by this "*wash*" step was less than the 100% required in the

claim (see statement of grounds of appeal by the patent proprietor, point 5.33). However, the board agrees with the opponent that D2 discloses use of the filtrate of the ultrafiltration step, and not of the concentrated enzyme solution, for washing the solid phase (column 3, lines 63 to 65). The dilution factor is thus not a distinguishing feature.

11. The patent proprietor further argued that the enzyme in the method of D2 was not in "*precipitated form*" in the sense of claim 1, but represented soluble enzyme adhered to biomass. Therefore the form in which the enzyme was present was a distinguishing feature of the claimed method over D2. Moreover, the washing step disclosed in D2 was not to be equated with solubilisation because it merely washed out the bound enzyme, but did not solubilise it.

12. The board does not agree. Document D2 discloses the separation of the fermentation broth into a first phase and a second phase. The latter is called "*Biomasse*" ("*biomass*") and contains insoluble accompanying compounds or solids, cell debris and/or intact bacteria (see column 2, lines 46 to 49: "*unlösliche Begleit- oder Feststoffe, einschließlich der Zelltrümmer und/oder der intakten Bakterien, im Folgenden auch Biomasse genannt*"). This "*Biomasse*" is said to still contain residual amounts of valuable substances ("*Wertstoffe*", i.e. enzyme, see column 3, lines 5 to 7). D2 further refers in column 1, lines 57 to 58, to: "*die durch Separation der Kultur-, bzw. Fermenterbrühe abgetrennten Feststoffe*" ("*the solids separated by separation of the culture or fermenter broth*") and in column 1, lines 62 to 64, to: "*darin wasserlösliche Wertstoffe, insbesondere extrazelluläre Wertstoffe herausgelöst*" ("*water-soluble valuable*

substances therein, in particular extracellular valuable substances, [can be] dissolved out"). The board concludes that the disclosure of D2 is not limited to enzyme bound to cell mass/insolubles, but includes all enzyme in insoluble form which ends up in the mostly solid phase.

13. Assuming, in adopting the patent proprietor's interpretation (see point 8. above), that "*enzyme in precipitated form*" did not include enzyme bound to biomass, the board therefore still agrees with the opposition division that D2, in referring to "*Feststoffkonzentrat*" from which "*Wertstoffe*" could be extracted or dissolved out, implicitly also discloses enzymes in precipitated form according to this narrow definition chosen by the patent proprietor (see points 60 to 60.3 in the decision under appeal).

14. The board therefore concludes that the form in which the enzyme is present in the fermentation broth is not a distinguishing feature.

15. The board also does not agree that D2 only discloses washing and not solubilising the enzyme. "*Washing*" in the common sense of the word refers to the transfer of unwanted material, e.g. contaminants, from a solid into the washing solution. In D2 the word "*waschen*" is, however, used in a different sense, namely, the extraction of valuable compounds, e.g. the enzyme, from a solid phase into the wash solution by solubilisation of these compounds (column 2, lines 60 to 64: "*Durch das Nachwaschen der abgetrennten Feststoffkonzentrate können darin wasserlösliche Wertstoffe, insbesondere extrazelluläre Wertstoffe herausgelöst und der Wertstofflösung zugeführt werden.*"; "*By rewashing the separated solid concentrates, water-soluble valuable*

substances therein, in particular extracellular valuable substances, can be dissolved out and fed into the solution of valuable substances."). The German word "*herausgelöst*" ("*dissolved out*") makes this clear. This, however, is nothing other than the solubilisation referred to in the claimed method and as performed in the examples of the patent.

16. Since document D2 not only shares most of the steps with the claimed method, but also has the same purpose of improving the yield of enzyme from a fermentation broth, it is a suitable starting point for assessing the inventive step of claim 1.

Difference, technical effect and objective technical problem

17. As outlined above, none of the form of the enzyme ("*precipitated*"), the dilution factor ("*100-2000% (w/w)*"), or the step of solubilisation are distinguishing features of the claimed method over D2.

18. The only remaining differences are therefore the pH ("*below 6.0*") and the method step in which it is adjusted.

19. The opposition division in its decision found that the pH in the solubilisation step did not necessarily have to be adjusted after separation of the two phases, but could also be adjusted before because "*the claims of the main request do not exclude an adjustment step of the fermentation broth (e.g. [0040])*" (point 59.1 of the decision under appeal).

20. The board agrees that the claim wording ("*method comprising the steps*"), supported by the description of the patent in paragraph [0040], allows a pH adjustment

step before separation ("*Before the separation method according to the invention the fermentation broth may be subjected to one or more pretreatment steps, such as dilution, adjusting pH* [...]"). This, however, does not negate the fact that the solubilisation step which takes place after the first separation step requires "*adjusting the pH*" as an active step. Adding the filtrate to the sediment, wherein both have the same pH because they derive from the same fermentation broth, as disclosed in D2, cannot be considered an adjustment of the pH after separation of the two phases (see also the patent proprietor's statement of grounds of appeal, points 5.25 to 5.32).

21. The method of claim 1 thus differs from the method in D2 in that the pH of the second phase is adjusted to below 6.0 after the separation of the two phases.

22. The patent proprietor argued that the claimed method saved resources, e.g. water and chemicals, compared to methods in the prior art. Such an effect has, however, not been shown in comparison to the method of D2 which, by re-using the filtrate to extract residual enzyme, already operates in a resource-saving manner. No other improvement, e.g. in the degree of solubilisation at pH 6.0, has been shown in comparison to the method disclosed in D2 either.

23. The objective technical problem can thus be formulated as the provision of an alternative method for recovering a protease in precipitated form from a fermentation broth.

Obviousness

24. The skilled person would have considered document D6 to be relevant to the solution of this problem because it discloses methods for the solubilisation and recovery of proteases in precipitated form, in particular in crystalline form. D6 furthermore discloses conditions for solubilising specific proteases, e.g. subtilisin 309 and variants thereof, and mentions "*SAVINASE™*" (see page 2, line 17) which is a protease also referred to in D2 (see column 2, line 39). The conditions for solubilisation are for example provided in claim 1 of D6:

> "*a) diluting the fermentation broth 100-2000% (w/w);*
> *b) adding a divalent salt; and*
> *c) adjusting the pH value of the fermentation broth to a pH value below pH 5.5*"

25. It is of relevance to consider whether the skilled person faced with the objective technical problem would have applied the pH adjustment step, which in D6 targeted the whole fermentation broth, to the solid phase of D2, i.e. after phase separation.

26. The patent proprietor argued that, in view of the disclosure of D6, the skilled person would have adjusted the pH of the entire fermentation broth and not just that of the solid phase after separation.

27. The opponent argued that there was no reason for the skilled person not to adjust the pH to below 5.5 after separation of the two phases.

28. The opponent was of the opinion that "*solubilization* 'in the absence' *(or at least with low amounts) of the*

liquid part of the fermentation broth is routinely applied in protease purification and thus general knowledge in the art" (opponent's statement of grounds of appeal, page 40, penultimate paragraph). Solubilisation of the solid phase (retentate, sediment, spin-down fraction) is routinely applied in enzyme purification as evidenced by a number of documents (see e.g. document D3, page 9, lines 7 to 12, and page 19, lines 18 to 20; document D4, examples and claim 1; document D5, column 4, lines 29 to 44; and document D10, page 224, Materials and Methods, third paragraph).

29. The board thus finds it to be part of the common general knowledge of the skilled person that solubilisation can be performed after separating the precipitated protease into a mostly solid phase. This is also done in D2 itself by mixing the centrifugation sediment with the ultrafiltration filtrate, albeit without adjusting the pH.

30. The skilled person, however, would have learned from document D6 that lowering the pH increased solubilisation of the precipitated protease (see tables 1 and 2 therein). When aiming at an alternative method for recovering enzyme in precipitated form, the skilled person would thus have had no reason not to apply the solubilisation conditions of D6 after separation of the two phases.

31. In view of the good solubilisation results and activities reported in D6 (see e.g. tables 1 and 2), the skilled person also had a reasonable expectation of success when it came to recovering active protease from the sediment in this way. The board notes, moreover, that the claim does not require a particular degree of recovery of active enzyme.

32. The skilled person, faced with the objective technical problem formulated above and starting from document D2, would have applied the solubilisation conditions of D6 after separation of the two phases and would thus have arrived at the claimed method. The method of claim 1 thus lacks an inventive step (Article 56 EPC).

Auxiliary request 1 - claim 1
Inventive step (Article 56 EPC)

33. The claim differs from claim 1 of the main request in that the divalent salt and its amount are further defined. It was undisputed that these specific conditions are disclosed in D6 (see examples 1 and 2).

34. The subject-matter of claim 1 lacks an inventive step for the same reasons as the subject-matter of claim 1 of the main request.

Auxiliary request 2
Admission (Article 12(4) and (6) RPBA)

35. The board admitted the request for reasons of procedural efficiency, as the deletion of dependent claim 10 appeared only to solve outstanding issues and not to create any new ones. Moreover, in view of the findings on inventive step for this request (see below), the opponent did not appear to be disadvantaged by such admittance.

Inventive step (Article 56 EPC) - claim 1

36. The claim differs from claim 1 of auxiliary request 1 in that "*before the first separation step, the fermentation broth is subjected to dilution as a*

pretreatment step". This additional feature does not provide a further difference because the method in D2 discloses diluting the fermentation broth by adding a $CaCl_2$ solution before the separation (e.g. centrifugation) step (see example of D2, column 3, lines 49 to 51: "*Eine Fermentercharge, die Protease als Wertstoff enthielt, wurde mit einer 30%igen $CaCl_2$-Lösung auf den pH-Wert von 7,5 eingestellt.*"; "*A fermenter batch containing protease as a valuable substance was adjusted to a pH value of 7.5 using a 30% $CaCl_2$ solution.*"). The patent proprietor has also not proposed any particular effect associated with such dilution pretreatment of the fermentation broth.

37. The subject-matter of claim 1 lacks an inventive step for the same reasons as the subject-matter of claim 1 of auxiliary request 1 and the main request.

Auxiliary request 3 - claim 1
Inventive step (Article 56 EPC)
Document D4 as the closest prior art

38. The opponent cited D4 as a suitable starting point for assessing inventive step of the subject-matter of claim 1 (see point 8.1.1. of the statement of grounds of appeal). With regard to the main request, the opposition division had rejected D4 as a starting point because in its view the document was primarily concerned with the (purposive) precipitation of the protein of interest while precipitation was considered a disadvantage in the patent (see decision under appeal, point 41.1). The decision under appeal instead started from the disclosure of D2 when assessing the inventive step of the main request and auxiliary requests 1 to 3.

39. The patent proprietor also rejected D4 as a starting point for assessing inventive step of the subject-matter of claim 1 of any of the requests, arguing that the document was directed to methods of separating enzymes in a mixture via selectively retaining one enzyme with a cross-flow membrane. D4 was not primarily concerned with the isolation of an enzyme present in precipitated form in a fermentation broth (see Annex A, point 6.14, filed with the patent proprietor's statement of grounds of appeal, and its reply to the opponent's statement of grounds of appeal, points 8.28 to 8.37). The patent proprietor further argued that the most important test for selecting the closest prior art was that the document was directed to the same technical problem. This was not the case for D4, with precipitation of an enzyme being the purpose of the document. In attempting to improve solubilisation of a precipitated enzyme, it was unreasonable to argue that the skilled person would turn to a document that related to purposefully carrying out the opposite process.

40. The board, in addition to its principal reasoning with regard to the choice of the "*closest prior art*" (see point 2. above), disagrees with the argument that D4 discloses a method which is the opposite process to the one claimed. D4, as shown below, discloses the same two principal steps as the claimed method, namely separation of a fermentation broth into two phases followed by solubilisation of a precipitated protein in the phase which contained mostly solids.

41. The claimed method does not exclude the "*purposive*" precipitation of an enzyme in the fermentation broth as alleged by the patent proprietor. In contrast, the description of the patent even refers to prior art

methods (see paragraph [0004] of the patent, referring to WO 93/13125; D3 in the present proceedings) which disclose such "*purposive*" precipitation and states that "*any of these methods for fermenting the microorganisms can be used together with the methods of the present invention*" (see paragraph [0019] of the patent). Also the "*DETAILED DESCRIPTION OF THE INVENTION*" of the patent starts with the explanation that the "*invention relates to the primary separation of a fermentation broth where part of the desired fermentation product is present in solid form such as in crystalline or amorphous form*" and "*what is important is that a significant part of the desired product is in solid form*" (paragraph [0014] of the patent). The claimed method cannot therefore be regarded as being limited to methods in which precipitation only occurs during fermentation and not by any additional steps or adjustment of the conditions after fermentation. This interpretation is further supported by paragraph [0040] of the patent, which refers to "*pretreatment steps*" which might be carried out before separation.

42. D4 discloses methods "*relating to the recovery of a protein of interest from a culture solution*" (see paragraph [0024]) and the examples of this document relate to "*recovery of protein from fermentation broth*".

43. D4 also relates to methods in which conditions are applied during the first separation step which "*maintain the protein of interest in a form where the apparent molecular weight of the protein prevents passage through the membrane*" (D4, paragraph [0050]), i.e. methods where already existing higher molecular weight structures of the enzyme are maintained by a first set of conditions. This is also apparent from

paragraph [0047] of D4 which states that "[i]*n some cases, the method allows the purification and recovery of such proteins directly from fermentation broth using only a single membrane cross-flow membrane filtration apparatus.*" Also in the examples (e.g. examples 1, 3 and 6), the first condition, which causes the protein to precipitate, is set in the fermentation broth as early as prior to the filtration step, i.e. not only on the filtration membrane.

44. In conclusion, the board sees no reason why D4 could not serve as a starting point for assessing inventive step.

45. D4 uses cross-flow filtration for separation of the protein of interest (see paragraph [0024]). The board notes that filtration is a method envisaged for the first separation step in the claim (see paragraph [0047] of the patent). The method of D4 is exemplified with hydrophobin and α-amylase, but "*can be applied to other enzymes*" (paragraph [0055] of D4). The board further notes that the examples of the patent also include other enzymes such as α-amylase and muramidase (see examples 4 and 5), i.e. the originally claimed method was not limited to protease (see claims 1 and 5 of the application as filed).

46. Claim 1 of D4, which outlines the general steps of the method, reads:

> "*1. A method for recovering a protein of interest from a culture solution using cross-flow membrane filtration, comprising: subjecting a culture solution comprising a protein of interest to cross-flow membrane filtration using a first membrane under a first set of conditions under which the apparent molecular weight of the protein of*

interest is greater than its molecular weight that cause the protein of interest to be retained as retentate to allow purification, concentration, and/or buffer exchange of the protein of interest; exposing the protein of interest retained by the first cross-flow membrane to a second cross-flow membrane under a second set of conditions under which the apparent molecular weight of the protein of interest is reduced such that it that passes through the second membrane as filtrate to allow purification and/or recovery of the protein of interest; wherein the first membrane and the second membrane are the same type of cross-flow filtration membrane and have a pore size that provides a molecular weight cut-off that is greater than the molecular weight of the protein of interest; characterized in that the first set of conditions causes the protein of interest to form multimers, to aggregate, to crystallize, to precipitate, to form a gel, or combinations thereof."

47. In other words, D4 discloses the separation of a protein which is present in a higher molecular weight form (e.g. crystallised) in a fermentation broth together with cells and cell debris (see D4, dependent claim 5) as "*retentate*" on a membrane. This "*retentate*" corresponds to the second phase in present claim 1. The liquid comprising lower molecular weight forms flows through the membrane and thus corresponds to the first phase in present claim 1. Applying appropriate conditions to the second phase ("*retentate*") reduces the molecular weight of the protein, i.e. disaggregates or solubilises it, such that the protein can flow through a second membrane while cells and cell debris are optionally retained (see D4, dependent claim 6). It is also clear from the examples of D4 that reduction of

the apparent molecular weight of the enzyme results in its solubilisation because the enzyme is found in the "*permeate*" of the second filtration (see e.g. paragraphs [0071], [0076], [0088]). "*Crystals*" and "*crystallize*" are mentioned in a list of higher molecular weight structures (see claim 1 and paragraphs [0014], [0032], [0048] and [0049] of D4), but only a selection from a single list is required to arrive at this embodiment.

Difference, technical effect and objective technical problem

48. The claimed method thus differs from the method disclosed in D4 in the enzyme which is recovered, i.e. the protease as defined in the claim, and in the conditions for solubilisation of this enzyme.

49. There is no particular technical effect associated with the aforementioned differences that is apparent from the application as filed, nor has one been invoked by the patent proprietor.

50. The objective technical problem can thus be formulated as the provision of a method for recovery of a further enzyme from fermentation broth.

Obviousness

51. The skilled person faced with this technical problem would have found the protease subtilisin 309 and its variants together with conditions for their solubilisation in D6. It was undisputed that subtilisin 309 falls under the definition of the protease according to claim 1. D6 furthermore indicates that a part of the enzyme was in crystalline form in the fermentation broth (examples 1 and 2: "*protease*

(subtilisin 309) crystals" and "*protease (subtilisin 309 variant) crystals*"). In accordance with the disclosure of D4, the skilled person would thus have applied the fermentation conditions of D6 (e.g. no dilution, pH 6.9 or pH 7.4, no calcium added) in order to maintain the enzyme in crystalline form during the first step of separation (see D4, paragraph [0050], "*the conditions maintain the protein of interest in a form where the apparent molecular weight of the protein prevents passage through the membrane*"). D6 further discloses how the subtilisin protease can be solubilised, namely by diluting 100-2000% (w/w), addition of $CaCl_2$ and adjustment of the pH value to below pH 5.5 (see claim 1 of D6 and examples). The skilled person would thus have applied these conditions to the retentate comprising the protease in crystalline form because D4 already proposes adapting conditions by modifying the salt concentration, precipitant concentration and the pH (see paragraph [0027] and claim 10). It is part of the common general knowledge of the skilled person that solubilisation can be performed in the absence of at least part of the liquid part of the fermentation broth, i.e. after separating the protease into a mostly solid phase (see points 28. and 29. above). This is routinely done in enzyme purification (see e.g. D4 itself, examples and claim 1; D3, page 9, lines 7 to 12, and page 19, lines 18 to 20; D5, column 4, lines 29 to 44; and D10, page 224, Materials and Methods, third paragraph).

52. In view of the good solubilisation results and activities reported in D6 (see tables 1 and 2), when applying the method of D4 to the particular protease disclosed in D6 the skilled person also had a reasonable expectation of success in recovering active protease from the retentate. The board notes that the

claim does not require a particular degree of recovery of active enzyme.

53. The board concludes that the skilled person, faced with the above-mentioned objective technical problem, would have arrived at the claimed method when starting from the disclosure of D4 and combining it with the disclosure of D6. The claimed method lacks an inventive step within the meaning of Article 56 EPC.

Auxiliary request 4 - claim 1
Amendments (Article 123(2) EPC)

54. The opponent objected *inter alia* that the introduction of the feature "*wherein the solubilization step takes place in the absence of the liquid part of the fermentation broth*" extended the subject-matter of the claim beyond the content of the application as filed.

55. The passage on page 3, lines 29 to 31, of the description as filed, which was cited by the patent proprietor as basis in this regard, reads as follows: "*the solubilisation of the desired product can take place in the absence of at least part of the liquid part of the fermentation broth containing the majority of these disadvantageous components*". Two sub-features of this passage, namely "*of at least part*" and "*containing the majority of these disadvantageous components*", are not present in claim 1.

56. In its decision, the opposition division found that the claim did not add subject-matter because the skilled person would have understood from this passage and the application as a whole that it would be beneficial if the liquid part containing the majority of the disadvantageous components was absent. Furthermore, the

skilled person would have understood that the absence of the whole liquid part was more favourable than absence of only a part thereof. An additional pointer was provided by the examples, which showed separation of the fermentation broth into a solid and a liquid fraction. This reflected the absence of all of the liquid part. Furthermore, the feature that said liquid part contained the majority of the disadvantageous components was considered inherent (see decision under appeal, points 75 to 77.2).

57. The board does not agree with this reasoning. It is not sufficient that subject-matter created by an amendment might have been considered beneficial or favourable by the skilled person having regard to the application as filed, but rather whether a skilled person would derive the subject-matter directly and unambiguously, using common general knowledge, and seen objectively and relative to the date of filing, from the whole of the application as filed (so-called gold standard, see e.g. decision G 2/10, OJ EPO 2012, 376).

58. To assess whether no subject-matter is added by an amendment of a claim, the board considers it necessary to first establish the meaning of the amendment within the context of the claim, i.e. to construe the subject-matter of the claim after amendment from the perspective of the skilled person. Only then is it possible to compare the subject-matter of the amended claim with the disclosure of the application as filed to see whether the former is directly and unambiguously derivable from the latter.

59. The board, however, cautions that when assessing amendments under Article 123(2) EPC (or Article 76(1) EPC, as the case may be) circular

reasoning has to be avoided, in particular when a claim feature in itself imparts clear, credible technical teaching (see e.g. T 1018/02, Reasons 3.8). Such "*primacy of the claims*" (see T 1473/19, Reasons 3.16.1 and 3.16.2) has also been confirmed in decision G 1/24 which found that "*The claims are the starting point and the basis for assessing the patentability of an invention under Articles 52 to 57 EPC.*" (see Reasons, 12, and Order).

60. Interpreting the claims by overly relying on the description would also carry the risk of reading into the amended subject-matter features which are only disclosed in the application as filed, but no longer present in the amended subject-matter, and would thus run contrary to the prohibition to extend the subject-matter beyond the content of the application as filed (Articles 123(2) and 76(1) EPC).

61. Claim 1 as amended (omissions by the board) reads:
"1. A method for recovering one or more enzyme from a fermentation broth, where the one or more enzyme is present in precipitated form in the fermentation broth, the method comprising the steps of:
a) a first separation step separating the fermentation broth in a first phase and a second phase, wherein the first phase comprises supernatant, the one or more enzyme in soluble form and optionally cells and cell debris, and the second phase comprises the one or more enzyme in precipitated form, cells and cell debris; and
b) a solubilization step where the one or more enzyme in precipitated form in the second phase is solubilized,
wherein the solubilization step comprises: [...], and
wherein the solubilization step takes place in the absence of the liquid part of the fermentation broth,

wherein the one or more enzyme is selected among proteases [...]."

62. The claimed method involves separation of the fermentation broth into two phases. The first phase comprises supernatant and the one or more enzyme in soluble form. The first phase thus comprises liquid because "*supernatant*" is commonly understood as the liquid resulting from the separation of liquids and solids (see also the patent proprietor's reply to the opponent's statement of grounds of appeal, point 4.4) and "*enzyme in soluble form*" necessarily also requires the presence of a liquid. The first phase optionally further comprises solids, such as cells and cell debris. The second phase comprises solids (precipitated enzyme, cells and cell debris). The word "*comprises*", however, indicates that further components (solid and liquid) of the fermentation broth may be present in both phases. Finally the claim requires that the solubilisation takes place "*in the absence of the liquid part of the fermentation broth*".

63. The parties differed in their interpretation of what "*in the absence of the liquid part of the fermentation broth*" meant.

64. During the oral proceedings, the patent proprietor argued that "*absence*" should not be interpreted literally because complete absence of liquid, i.e. a dry phase, would make no technical sense and was also not disclosed in the application as filed. The opponent, in contrast, considered that it was not clear what absence of the liquid part meant and that the opposition division had chosen different contradictory interpretations for its reasoning on added subject-

matter and clarity (see statement of grounds of appeal, points 5.3).

65. The patent proprietor further argued that the skilled person would equate the liquid part with the "*first phase*" or the "*supernatant*" comprising the one or more enzyme in soluble form (see its reply to the opponent's appeal, points 4.1 to 4.12). "*Absence of the liquid part of the fermentation broth*" therefore meant absence of the first phase. The opposition division apparently agreed with this view in the decision under appeal in the context of clarity (see point 79). The opponent counter-argued that neither the claim nor the description equated the "*liquid part*" with the "*first phase*" or with the "*supernatant*".

66. The board interprets the feature "*in the absence of the liquid part of the fermentation broth*" as follows. The claim wording does not provide any basis to read the "*liquid part*" in step b) as being equal to "*supernatant*" or "*first phase*" mentioned in step a) of the claim. Claim 3, which is dependent on claim 1, directly contradicts such equivalence by stating "*the first phase comprises at least 60 % of the liquid part of the fermentation broth, e.g. at least 70%, e.g. at least 80% of the fermentation broth*". The description also contains no indication that the "*liquid part of the fermentation broth*" was to be read as the "*supernatant*" or the "*first phase*". Rather, the description contains several instances where the liquid part of the fermentation broth is distributed over both phases (see e.g. paragraph [0042]: "*a first fraction comprising a liquid part of the fermentation broth*"; paragraph [0043]: "*Both the first fraction and the second fraction may comprise liquid part of the fermentation broth*"; examples 1 to 3).

67. The term "*absence*" is a clear technical term indicating that something is not present. The degree of "*absence*" of a certain compound in a composition can, however, depend on the methods of producing the composition and the limits of detection, i.e. 100% absence might not be achievable.

68. Not even consulting the description would lead to a different interpretation. The term "*absence*" is mentioned only once in the description, namely in paragraph [0017] in the context of "*absence of at least part of the liquid part*". This is consistent with the skilled person's general understanding when consulting the application as a whole that the liquid part of the fermentation broth is distributed over both phases during the methods of the invention. The passage therefore provides no guidance as to how to interpret the term "*absence*" of the (whole) liquid part.

69. The board therefore interprets "in the absence of the liquid part of the fermentation broth" to mean what it says, namely, that all of the liquid part of the fermentation broth is absent.

70. This interpretation is also technically sensible in the context of the claim because the claim leaves open the possibility of removing residual liquid from the second phase before solubilisation since the method "*comprises*" a first separation step and a solubilisation step, but may contain further steps, such as a drying step of the second phase before solubilisation. Even though the patent does not disclose such a further step, it would not be inconsistent with the disclosure of the patent.

71. The board concludes that the claim as read by the skilled person having consulted the description (but not overruling the clear meaning of the wording of the claim) refers to a method of separation of a fermentation broth into two phases and the solubilisation of enzyme in precipitated form present in the second phase in the absence of the whole liquid part of the entire fermentation broth.

72. The issue as to whether the application as filed discloses such a method is addressed below.

73. The only passage provided as a basis for the feature under dispute is on page 3, lines 29 to 31, of the application as filed, which states "*solubilisation of the desired product can take place in the absence of at least part of the liquid part of the fermentation broth containing the majority of these disadvantageous components*". The patent proprietor argued that "*at least part*" comprised two alternatives: either (i) some or (ii) all of the liquid part of the fermentation broth was absent during the solubilisation step (see reply to the opponent's appeal, point 5.7). The latter was a preferred embodiment as evident from other passages in the application as filed which showed that solubilisation was preferably carried out in the absence of the first phase of the method (see page 3 and the examples). While the board agrees that "*in the absence of at least part*" in isolation could be interpreted as referring to (i) some or (ii) all of the liquid part, the second alternative would not have been considered by the skilled person having regard to the remainder of the application as filed.

74. Firstly, and as already established when construing the amended claim above, the "*liquid part of the*

fermentation broth" cannot be equated with the "*supernatant*" or the "*first phase*" mentioned in the claim.

75. Secondly, the passages referring to "*liquid part of the fermentation broth*" do not disclose the absence of all of the liquid part:
- page 11, line 30: "*a first fraction comprising a liquid part of the fermentation broth*", i.e. a liquid part, not the (whole) liquid part
- page 12, lines 1 and 2: "[b]*oth the first fraction and the second fraction may comprise liquid part of the fermentation broth*", i.e. the liquid part is present in both fractions
- page 12, lines 4 to 6: "[t]*he first fraction comprises at least 60 % of the liquid part of the fermentation broth, e.g. at least 70%, e.g. at least 80%, of the fermentation broth, whereas the second fraction comprises the reminder*", i.e. the first fraction contains a maximum of 80% of the liquid part, the remainder of the liquid part being in the second fraction

76. Thirdly, none of the examples shows solubilisation of protease in the absence of the entire liquid part of the fermentation broth, as it is commonly known that neither centrifugation nor filtration as described are suitable for removing all liquid:
- example 1B, page 22, lines 14 to 15, and example 2B, page 23, lines 23 and 24, state that "[t]*he separation was performed using a Custom designed centrifuge*"
- example 3B, page 24, line 23: "*separation by drum filtration*"

77. In conclusion, the passage on page 3, lines 29 to 31 does not, by referring to "*at least part of the liquid part*" in the context of the application as filed as a whole, disclose a method where a fermentation broth is separated into two phases wherein the second phase does not contain the (whole) liquid part of the fermentation broth during solubilisation (see points 62. to 71. above).

78. The application as filed thus does not disclose step b) of the claimed method in the absence of the liquid part of the fermentation broth. The subject-matter of claim 1 thus extends beyond the content of the application as filed (Article 123(2) EPC).

Auxiliary request 5
Admittance (Article 12(4) and (6) RPBA)

79. The board admitted the request for reasons of procedural efficiency in order to consider this request with regard to substance. As the substantial findings with regard to this request (see below) rendered the request not allowable, the opponent was not adversely affected.

Amendments (Article 123(2) EPC) - claim 1

80. The feature "*wherein the solubilization step takes place in the absence of the liquid part of the fermentation broth*" is also present in claim 1 of this request. Claim 1 of this request does not fulfil the requirements of Article 123(2) EPC for the same reasons as claim 1 of auxiliary request 4.

Order

For these reasons it is decided that:

1. The decision is set aside

2. The patent is revoked.

The Registrar: The Chair:

I. Aperribay M. Blasi

Decision electronically authenticated