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**Datasheet for the decision
of 28 January 2021**

Case Number: T 0518/17 - 3.3.04

Application Number: 08717479.3

Publication Number: 2125865

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C12N15/31

Language of the proceedings: EN

Title of invention:

Solubilization of protease crystals in fermentation broth

Patent Proprietor:

Novozymes A/S

Opponent:

BASF SE

Headword:

Solubilising subtilisin crystals in a fermentation broth/
NOVOZYMES

Relevant legal provisions:

EPC Art. 100(b), 83
RPBA Art. 12(4)

Keyword:

Sufficiency of disclosure - (no)

Evidence submitted with statement of grounds of appeal -
admitted (yes)

Decisions cited:

T 0409/91

Catchword:



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Case Number: T 0518/17 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 28 January 2021

Appellant: BASF SE
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted on 23 December 2016 rejecting the opposition filed against European patent No. 2 125 865 pursuant to Article 101(2) EPC**

Composition of the Board:

Chair B. Claes
Members: R. Morawetz
M. Blasi

Summary of Facts and Submissions

- I. The appeal of the opponent ("appellant") lies from the opposition division's decision rejecting the opposition to European patent No. 2 125 865 ("the patent"). Accordingly, the patent proprietor is the respondent in these proceedings.

The patent, entitled "*Solubilization of protease crystals in fermentation broth*", derives from European patent application No. 08 717 479.3 with a filing date of 6 March 2008, and claims priority from European patent application No. 07 104 220.4, filed on 15 March 2007.

Claim 1 of the patent as granted reads:

"1. A method of solubilizing protease crystals and/or protease precipitate in a fermentation broth comprising

- 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 5.5,
- wherein the protease is a subtilisin."

- II. One opposition was filed against the patent in its entirety. The opposition proceedings were based, *inter alia*, on the ground for opposition under Article 100(b) EPC.

- III. The following documents are referred to in this decision:

D2 WO 93/13125 (8 July 1993)

- D4 US 6,316,240 (13 November 2001)
- D7 WO 03/050274 (19 June 2003)
- D29 Experimental report, filed on 30 September 2016
- D30 Experimental data, filed on 18 October 2016
- D42 WO 2004/003216 (8 January 2004)
- D43 EP 0 396 608 B1 (3 April 1996)
- D44 WO 00/37599 (29 June 2000)
- D45 WO 02/00907 (3 January 2002)
- D46 WO 96/23873 (8 August 1996)
- D47 Experimental report, filed with the statement of grounds of appeal
- D48 Experimental report, filed with the statement of grounds of appeal
- D49 WO 2009/152176 (17 December 2009)
- D50 Classical nucleation theory (https://en.wikipedia.org/wiki/classical_nucleation_theory) (29 June 2017)
- D53 H.A. Herrmann et al., in *Enzymes in Detergency* (1997), J.H. van Ee, O. Misset and E.J. Baas, editors, pages 251 to 297

- IV. The opposition division admitted document D29 into the proceedings, while document D30 was not admitted. During the opposition proceedings, the appellant had argued that it was not possible to obtain subtilisin crystals using the methods described in the patent. The respondent's respective counter-arguments were that documents D2, D4 and D7 showed subtilisin crystals or precipitate and that document D29 showed that crystals of subtilisin could be formed if the conditions used in the examples of the patent were met.
- V. With their statement of grounds of appeal, the appellant filed, *inter alia*, documents D42 to D48 and submitted arguments that the patent did not disclose the invention in claim 1 as granted in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.
- VI. In reply to the statement of grounds of appeal, the respondent: requested that the appeal be dismissed, i.e. that the opposition be rejected, hence that the patent be maintained as granted (main request); submitted arguments with respect to, *inter alia*, sufficiency of disclosure; and filed sets of claims of auxiliary requests 1 to 8 (although claims of an auxiliary request 9 were also announced, a set of claims was omitted from the reply) and documents D49 and D50. The other documents filed by the respondent are not mentioned in this decision as they were not relied upon in support of the respondent's arguments with regard to sufficiency of disclosure.

Claim 1 of auxiliary request 1 differs from claim 1 of the main request (see section I. above) in that step c) reads "*adjusting the pH value of the fermentation broth to a pH value between 2.0 and 5.0*".

Claim 1 of auxiliary request 2 differs from claim 1 of the main request in that it includes the feature "*and the divalent salt is a calcium salt and/or a magnesium salt added at a concentration of 0.01-5 % relative to the diluted fermentation broth*" at the end.

Claim 1 of auxiliary request 3 combines the amendments made to claim 1 of auxiliary request 1 and those made to claim 1 of auxiliary request 2.

Claim 1 of auxiliary request 4 differs from claim 1 of the main request in that it includes the feature "*the protease crystals and/or the protease precipitate are obtained from a microorganism, and the microorganism is a Bacillus cell*" at the end.

Claim 1 of auxiliary request 5 differs from claim 1 of auxiliary request 1 in that in step a) the feature "*with water, an ultrafiltration permeate or a mixture of water and ultrafiltration permeate*" is introduced after the feature "*diluting the fermentation broth*".

Claim 1 of auxiliary request 6 combines the amendments made to claim 1 of auxiliary request 2 and those made to claim 1 of auxiliary request 5.

Claim 1 of auxiliary request 7 combines the amendments made to claim 1 of auxiliary request 3 and those made to claim 1 of auxiliary request 5.

Claim 1 of auxiliary request 8 reads as follows:

"1. A method of solubilizing protease crystals and/or protease precipitate in a fermentation broth comprising

- a) diluting the fermentation broth with water, an ultrafiltration permeate, or a mixture of water and ultrafiltration permeate 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,

wherein the protease is a subtilisin, wherein the protease crystals and/or the protease precipitate are obtained from a microorganism, and the microorganism is a *Bacillus* cell."

VII. By letter of 11 September 2017, the respondent submitted a set of claims of auxiliary request 9 which had been omitted from their earlier filed reply (see section VI.).

Claim 1 of auxiliary request 9 reads as follows:

"1. A method of solubilizing protease crystals and/or protease precipitate in a fermentation broth comprising

- a) diluting the fermentation broth with water, an ultrafiltration permeate, or a mixture of water and ultrafiltration permeate 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,

wherein the protease is a subtilisin belonging to EC 3.4.21.62, wherein the protease crystals and/or the protease precipitate are obtained from a microorganism, and the microorganism is a *Bacillus* cell."

- VIII. The board summoned the parties to oral proceedings in line with their corresponding requests, and issued a communication under Article 15(1) RPBA 2007 informing them of its preliminary opinion with respect to the construction of claim 1 of the main request and sufficiency of disclosure of the claimed invention.
- IX. In response, both parties provided further arguments with respect to sufficiency of disclosure of the claimed invention. The respondent filed document D53.
- X. With the consent of both parties, the oral proceedings were held by videoconference. Admittance of documents D42 to D48, which were filed by the appellant in support of their case in relation to sufficiency of disclosure, was a matter of dispute between the parties. The board decided on admittance during the oral proceedings. Accordingly, documents D42 to D48 were not excluded from the appeal proceedings (Article 12(4) RPBA 2007). At the end of the oral proceedings, the chair announced the board's decision.
- XI. The appellant's arguments are summarised as follows:

*Admittance of documents D42 to D48
(Article 12(4) RPBA 2007)*

Documents D42 to D48 were *prima facie* relevant to sufficiency of disclosure.

Documents D42 to D46 were submitted in response to the opposition division's reasoning that the skilled person was able to provide a fermentation process that led to protease crystals. Documents D42 to D45 were cited in the patent and document D46 was cited in document D4.

The respondent had submitted document D29, the first document in the proceedings to show subtilisin crystals, shortly before the oral proceedings in opposition proceedings and the document had been admitted by the opposition division.

The appellant had filed document D30 during the oral proceedings in opposition proceedings in reaction to the respondent's filing of document D29, but the document had not been admitted into the proceedings.

Document D30 was re-submitted as document D47 in appeal. The experimental data in documents D47 and D48 were provided in response to the reasoning in the decision under appeal that the skilled person was well aware of how to set up a fermentation process that produced high protease yields and of the conditions that ultimately led to protease crystals and/or protease precipitate.

Main request - claim 1

Claim construction

The fermentation broth in step a) had to comprise subtilisin crystals and/or subtilisin precipitate, otherwise the purpose recited in the claimed method, i.e. solubilisation, could not be achieved.

Sufficiency of disclosure (Article 100(b) EPC)

It was indispensable for performing the claimed method to provide a fermentation broth comprising subtilisin crystals and/or subtilisin precipitate. Thus this step was an essential feature comprised in the method, even if the claim contained no explicit reference to the

feature.

Neither the patent nor the common general knowledge provided guidance to the skilled person for obtaining a fermentation broth comprising subtilisin crystals due to high yields of the enzyme.

Teaching of the patent

The patent addressed the presence of protease crystals caused by high yields of the enzyme in fermentation broths (paragraph [0002]).

The patent was silent on the technical measures in the fermentation process of subtilisin proteases that led to such high yields. The examples of the patent did not describe the fermentation method, and the patent did not provide any information on how to obtain subtilisin crystals in a fermentation broth.

Document D42, cited in paragraph [0021] of the patent as providing suitable fermentation conditions, did not disclose conditions that led to the formation of crystals in a fermentation broth.

The references cited in the examples of the patent for the subtilisin, documents D43 and D44, and for the *Bacillus* species used, document D45, did not disclose fermentation conditions that led to the formation of crystals in a fermentation broth. In each of these documents, enzyme activity was determined in the supernatant of the untreated culture broth (see document D43, page 12; document D44, page 32; document D45, page 50, line 1).

Common general knowledge of the skilled person

The skilled person only knew fermentation broths comprising subtilisin in soluble form and measures which were applied to convert the soluble form of subtilisin into crystals and/or precipitate (see document D2, page 18, last paragraph and Example 3 on page 19; document D7, Example 1). Document D4 did not disclose how crystals were obtained in the fermentation broth, and the crystals obtained in the example were from amylase and not from subtilisin.

Document D49 taught that subtilisin was soluble in the fermentation process and additional measures were needed to actively precipitate the enzyme (see Example 7).

Document D53 disclosed that additional measures, i.e. addition of salts, were needed to crystallise proteases (see page 253, fourth paragraph).

Thus documents D49 and D53 did not describe the presence of crystals due to high fermentation yields or the measures to be taken to reach such high yields in fermentation that led to protease crystals.

Document D53 disclosed the potential tool box for means to increase protein yield. This included strain modifications and improvements to the fermentation process. Document D53 showed that yield improvement reached a plateau after seven years (see Figure 4), and therefore did not support speculation that routine techniques for improving the production strain might improve the protease yield to such a level that proteases crystallised.

The increase in yield was a relatively modern problem which had arisen as a result of years of optimisation and improvement in the production process and production microorganism. However, the exact nature of the optimisation and improvements that were needed to provide the increase in yield were not known to the skilled person. Thus a research project was needed to arrive at fermentation conditions resulting in spontaneous formation of subtilisin crystals.

Bacteria did not continuously produce enzyme during fermentation, but eventually stopped enzyme production. Longer fermentation alone did not guarantee supersaturation.

Arguments to the effect that the skilled person was able to artificially induce the formation of crystals in a fermentation broth failed to support sufficiency of disclosure as the invention concerned solubilising subtilisin crystals that had formed in the formation broth due to very high subtilisin yields. Indeed, the skilled person would not artificially induce crystal formation only to dissolve them again if the subtilisin could be separated from the sludge right away without artificially inducing the crystals.

Post-published experimental evidence

It was unknown how the subtilisin crystals disclosed in document D29 had been obtained.

Document D48 was provided in response to the reasoning in the decision under appeal that the skilled person was well aware of how to set up a fermentation process that produced high protease yields and of the conditions that ultimately led to protease crystals.

The experiments reported in document D48 had been performed to test the hypothesis that high yields were crucial for generating subtilisin crystals in a fermentation broth. Supplementing subtilisin protease failed to provide crystals, indicating that additional measures were needed to induce formation of crystals in the fermentation broth.

An undue burden was required to carry out the claimed invention, and the patent therefore did not meet the requirement of sufficiency of disclosure.

Auxiliary requests 1, 2, 3, 5, 6 and 7 - claim 1

Sufficiency of disclosure (Article 83 EPC)

The same reasoning as given for claim 1 of the main request applied.

Auxiliary requests 4, 8 and 9 - claim 1

Sufficiency of disclosure (Article 83 EPC)

The limitation to subtilisin crystals and/or subtilisin precipitate obtained from a *Bacillus* cell did not reduce the burden for the skilled person to reproduce the claimed invention. The same reasoning as given for claim 1 of the main request applied.

XII. The respondent's arguments are summarised as follows:

*Admittance of documents D42 to D48
(Article 12(4) RPBA 2007)*

The filing of documents D42 to D48 was not justified by the decision under appeal. In fact, the appellant had

not been taken by surprise by this decision.

Documents D42 to D46 could have been submitted sooner and were of little or no relevance.

Document D47 should not be taken into account in appeal because it related to amylase, not proteinase. The appellant had failed to explain why the experimental results disclosed in D48 had not been filed in the opposition proceedings.

Main request - claim 1

Claim construction

The fermentation broth referred to in step a) had to comprise crystals and/or precipitate.

Sufficiency of disclosure (Article 100(b) EPC)

There was no explicit step in the claim of providing the fermentation broth, so this was not a step that the skilled person was required to perform according to the invention as claimed.

The claimed invention started at the point where the skilled person was faced with the recovery of subtilisin from a fermentation broth in which it was present in crystalline or precipitated form. If there were no subtilisin crystals or precipitate of protease in the fermentation broth, then there was no need subsequently to solubilise protease crystals and/or protease precipitate.

The skilled person could determine that subtilisin crystals were present in the fermentation broth. The

skilled person was able to carry out the invention without undue burden given the disclosure in the patent and the common general knowledge. There was no evidence that the skilled person was unable to vary the relevant factors in order to carry out the invention. The appellant had not discharged their burden of proof in this respect.

Teaching of the patent

The patent disclosed repeatable examples and provided extensive disclosure in paragraphs [0027] to [0046] about how to put the invention into effect.

"Whether or not crystals or precipitate are formed depends not only on the [bacterial] strain but also on the components of the fermentation medium and conditions such as temperature" (respondent's reply to the appeal, point 4.4).

Common general knowledge of the skilled person

Although the patent did not explicitly disclose the fermentation conditions, these belonged to the common general knowledge of the skilled person.

The formation of crystals or precipitate of enzyme in the fermentation broth was well known in the state of the art (see document D2, page 6, line 34 to page 7, line 3; document D4, column 1, lines 22 to 26 and column 2, lines 4 to 11 and 17 to 19; document D7, abstract and Example 1).

The dramatic increase in yield mentioned in paragraph [0002] of the patent was the prevailing situation in the state of the art.

"For industrial enzyme producers, the presence of enzyme crystals and/or precipitate is a relatively modern problem that simply did not exist in the past and has arisen as a result of years of optimisation and improvement in the production process and production microorganism." (respondent's letter dated 21 January 2020, page 1, 4th paragraph).

Document D49 disclosed that the improvements in fermentation yield were industry-wide (see paragraph [0011]). Further evidence of this increase in yield could be found in document D53 (see page 253, 4th paragraph). This document represented common general knowledge.

Increases in yield led spontaneously to the presence of subtilisin in the fermentation broth in the form of crystals and/or precipitate. The fermentation yield could be raised above the solubility limit by optimising the production microorganism using routine biotechnology techniques that were available to the skilled person from common general knowledge, e.g. by introducing strong promoters, mRNA stabilisers and codon-optimised coding sequences.

The skilled person knew that crystals grow in supersaturated solutions. If a fermentation was run long enough, the solution would eventually become supersaturated.

If the fermentation yield was not already above the solubility limit of the subtilisin, the salt concentration and/or pH could be modified to ensure the presence of subtilisin in the form of crystals. Alternatively, the fermentation broth could be spiked by adding crystals and/or amorphous solid subtilisin.

Post-published experimental evidence

Document D29 confirmed that subtilisin crystals could be formed in a fermentation broth.

There were a number of possible reasons why crystals had not been observed in document D48. Seventy-two hours might have been too short to achieve supersaturation. Nucleation, which was relevant to crystal formation, was a stochastic process in which the likelihood of crystal formation in a sample was proportional to the number of nucleation sites (see document D50). No steps, such as stirring, had been taken in document D48 to enhance crystallisation, nor to optimise ionic strength to increase the chances of obtaining crystals or precipitate. The fermentation medium had not been modified to decrease the solubility of the subtilisin during fermentation. The fermentation broth could have been metastable, requiring a seed crystal to start crystal formation.

Auxiliary requests 1, 2, 3, 5, 6 and 7 - claim 1

Sufficiency of disclosure (Article 83 EPC)

The same reasoning as given for claim 1 of the main request applied.

Auxiliary requests 4, 8 and 9 - claim 1

Sufficiency of disclosure (Article 83 EPC)

Claim 1 had been limited to subtilisin crystals and/or subtilisin precipitate obtained from a *Bacillus* cell and was thus closer to the examples of the patent. The skilled person could obtain crystals from *Bacillus*

without undue burden.

- XIII. The appellant requested that the decision under appeal be set aside and the patent be revoked.

The respondent requested that the appeal be dismissed, or alternatively that the patent be maintained in amended form on the basis of one of the sets of claims of auxiliary requests 1 to 8, all submitted with the respondent's reply to the statement of grounds of appeal, or on the basis of the set of claims of auxiliary request 9, submitted by letter of 11 September 2017.

Reasons for the Decision

1. The appeal complies with Articles 106 to 108 and Rule 99 EPC and is admissible.
2. A new version of the Rules of Procedure of the Boards of Appeal (RPBA 2020 (hereinafter referred to as "RPBA"): OJ EPO 2020, Supplementary publication no. 1, III.2) entered into force on 1 January 2020. The transitional provisions are set out in Article 25 RPBA. In the case at hand, the statement of grounds of appeal was filed before 1 January 2020. Therefore the admittance of documents filed with the statement of grounds of appeal is governed by Article 12(4) RPBA 2007.

Admittance of documents D42 to D48 (Article 12(4) RPBA 2007)

3. Together with the statement of grounds of appeal, the appellant submitted documents D42 to D48, admittance of

which had been contested by the respondent.

4. Pursuant to Article 12(4) RPBA 2007, the board has discretion to hold facts or evidence filed with the statement of grounds of appeal inadmissible if they could have been presented in the proceedings before the opposition division.
5. Shortly before the date of the oral proceedings in opposition proceedings, the respondent had submitted experimental data including photomicrographs of subtilisin crystals (document D29). The opposition division admitted these data into the opposition proceedings. Experimental data submitted in reaction by the appellant at the oral proceedings (document D30) were however not admitted into the opposition proceedings (see section IV. above).
6. In the decision under appeal, the opposition division held that the appellant had failed to provide evidence supporting their submission that the invention could not be carried out without undue burden, while document D29 was held to demonstrate that crystals could be formed and solubilised in accordance with the examples of the patent.
7. The board considers the appellant's re-submission of the experimental data (document D30) at the earliest stage on appeal (as document D47) together with further supporting data (document D48) an appropriate and legitimate reaction to the above-mentioned developments in the opposition proceedings.
8. Documents D42 to D45 are all cited in the patent as describing fermentation conditions, and the subtilisin and the *Bacillus* species used in Examples 1 and 2,

respectively. Document D46 is cited for the fermentation conditions in document D4. The board considers the filing of documents D42 to D46 with the statement of grounds of appeal an appropriate response on the appellant's part to the finding in the decision under appeal that guidance for carrying out the claimed invention was provided in the examples of the patent.

9. The board further considers that the appellant's submissions do not raise a fresh case but aim at further supporting their case on sufficiency of disclosure without exceeding the legal and factual framework beyond that already discussed before the opposition division (see section IV. above).
10. In view of the above considerations, the board decided not to exclude documents D42 to D48 from the appeal proceedings (Article 12(4) RPBA 2007).

Main request (patent as granted) - claim 1

Claimed invention - claim construction

11. The claim is directed to a method of solubilising subtilisin crystals and/or subtilisin precipitate in a fermentation broth. The method comprises three explicit steps, denoted a), b) and c). In step a), the fermentation broth is diluted 100-2000% (w/w).
12. The fermentation broth is not characterised further in the claim. However, the parties concurred that, as a consequence of the purpose indicated in the claim ("*solubilizing protease crystals and/or protease precipitate ... wherein the protease is a subtilisin*") the skilled person will understand that the fermentation broth referred to in method step a) has to

comprise subtilisin crystals and/or subtilisin precipitate. The board concurs with this claim construction. Accordingly, the claimed method comprises as step a) diluting a fermentation broth that comprises subtilisin crystals and/or subtilisin precipitate.

Sufficiency of disclosure (Article 100(b) EPC)

13. In the decision under appeal, the opposition division had held that *"the formation of crystals and/or precipitates cannot be considered as a technical step of the method claimed, and thus the points raised by the O's [appellant] in respect of the alleged inability to obtain at least such crystals are of no relevance to sufficiency of disclosure, since these steps do not have to be carried out as part of the claimed invention."*
14. The board points out that the underlying purpose of the requirement of sufficient disclosure of an invention is to ensure that the exclusive right conferred by a patent should be justified by the actual technical contribution to the art (see decision T 409/91, OJ EPO 1994, 653: point 3.5 of the Reasons). A technical contribution to the art requires that the claimed invention can be realised by the skilled person at the effective date of the patent. In the present case, realisation of the method requires that a fermentation broth comprising subtilisin crystals or subtilisin precipitate be available to the skilled person (see point 12. above).
15. Therefore the provision of a fermentation broth comprising subtilisin crystals and/or subtilisin precipitate is a feature of the claim that has to be taken into consideration when assessing whether or not

the claimed invention is sufficiently disclosed. The board considers it irrelevant in this context that the claim does not explicitly require the provision of such a fermentation broth, since such requirement is a necessary consequence of the express language of the claim and thus implied.

16. Accordingly, the opposition division's reasoning (see point 13. above) cannot hold, and the respondent's arguments that it was enough for sufficiency of disclosure that the skilled person could determine whether or not crystals were present in the fermentation broth and that the claimed invention started at the point where the skilled person was faced with the recovery of subtilisin from a fermentation broth in which it was present in crystalline or precipitated form must fail.
17. The appellant maintained on appeal that the patent, taking into account the common general knowledge of the skilled person, provided no guidance on how to obtain a fermentation broth comprising subtilisin crystals without undue burden. For this reason, they submitted that the requirement of sufficiency of disclosure was not met.
18. According to established case law of the boards of appeal, a successful objection of sufficiency of disclosure presupposes that there are serious doubts, substantiated by verifiable facts. In *inter partes* proceedings the burden of proof is initially upon an opponent to establish, on the balance of probabilities, that a skilled person reading the patent, using common general knowledge, would be unable to carry out the invention (see Case Law of the Boards of Appeal, 9th edition 2019 ["CLBA"], section III.G.5.1.2c)). If

the opponent has discharged their burden of proof, the patent proprietor seeking to refute the conclusively established facts by way of counter-arguments bears the burden of proving the alleged facts (CLBA, section III.G.5.2.1).

19. In the following, the board considers in turn the teaching of the patent, the common general knowledge and the post-published experimental evidence relied on by the parties.

Teaching of the patent

20. The present invention lies in the field of industrial production of the enzyme subtilisin, a serine protease, through fermentation. Microorganisms producing the enzyme are fermented under conditions leading to the secretion of the subtilisin into the fermentation broth (see paragraphs [0020] to [0025] of the patent). Subsequently, the biomass and further insolubles, commonly referred to as sludge, are separated from the subtilisin in the fermentation broth by filtration or centrifugation (see paragraphs [0039] to [0041]). The patent states that "*[t]he fermentation yield of industrial proteases has increased dramatically over the passed [sic] years. The yield is now so high that more than 60% of the protease in the fermentation broth may be present as crystals and/or precipitate*" (see paragraph [0002]) and that it provides "*an improved method of solubilizing protease crystals and/or protease precipitate in a fermentation broth*" (see paragraph [0001]). The method aims at ensuring that all the subtilisin is in solution in the fermentation broth and can be separated from the sludge by known separation methods, such as centrifugation and

filtration (see paragraph [0044]).

21. The board concurs with the appellant that it can be inferred from paragraph [0002] of the patent that the claimed method is to be applied in particular in circumstances in which the presence of subtilisin crystals in the fermentation broth is the result of a "dramatic" increase in the fermentation yield.
22. Accordingly, the claimed invention is understood to relate, in one embodiment, to diluting a fermentation broth that comprises subtilisin crystals as a consequence of high fermentation yields.
23. The first issue addressed is whether or not the patent provides the skilled person with guidance for carrying out this embodiment.
24. Although the patent provides information on proteases, microorganisms and fermentation broths that can be used according to the invention in the section of the description entitled "*Detailed Description*" (see paragraphs [0005] to [0047]), no information is provided on how to obtain a fermentation broth comprising subtilisin crystals as a consequence of high fermentation yields.
25. As for the examples, Example 1 of the patent, entitled "*pH and calcium effect on solubilizing protease (subtilisin 309) crystals*", discloses that the "*subtilisin was subtilisin 309 as described in EP 0396 608 B1 [document D43 in the appeal proceedings]*" and that the "*Bacillus species was Bacillus licheniformis with copies of the subtilisin inserted as described in WO 02/00907 [document D45]*" (see paragraph [0049]). Next, the solubilisation

process is described as starting from a "fermentation broth".

26. Example 2 of the patent, entitled "*pH and calcium effect on solubilizing protease (subtilisin 309 v ariant) crystals*", discloses that the "*subtilisin was a variant of subtilisin 309 as described in WO 00/37599 [document D44]*" and that the "*Bacillus species was Bacillus licheniformis with copies of the subtilisin inserted as described in WO 02/00907 [document D45]*" (see paragraph [0056]). Again, the solubilisation process is described as starting from a "fermentation broth". Neither example discloses how the fermentation broth was obtained.
27. As for the publications cited in the patent (see paragraphs [0049] and [0056] and points 25. and 26. above), the board notes that these documents report that subtilisin enzyme activity was determined in the supernatant of the untreated fermentation broth and the enzyme was recovered from the supernatant after centrifugation or filtration of the fermentation broth (see document D43, page 12, lines 46 to 56; document D44, page 32, lines 27 to 32; page 45, line 25 to page 46, line 8; document D45, page 50, line 1).
28. The reference in these documents to the recovery of subtilisin from the liquid phase after centrifugation or filtration indicates to the skilled person that the enzyme is present in soluble form. Accordingly, the board concurs with the appellant that the publications cited in the patent do not disclose fermentation conditions that lead to the formation of subtilisin crystals in a fermentation broth. Document D42, referred to in the patent as disclosing a complex fermentation medium (see paragraph [0021]), likewise

does not disclose such fermentation conditions.

29. The board notes in this context that according to the respondent's own submissions "*whether or not crystals or precipitate are formed depends not only on the [bacterial] strain but also on the components of the fermentation medium and conditions such as temperature*". No information on any of these parameters that would lead to subtilisin crystals in the fermentation broth appears to be disclosed in the patent or in the state of the art cited in the patent.
30. The board concludes from the above that it is a verifiable fact that neither the patent nor the state of the art cited therein provide any guidance for the skilled person with respect to the technical measures to be applied in the fermentation process of subtilisin proteases to achieve such high yields that subtilisin crystallises spontaneously in the fermentation broth during fermentation.
31. Accordingly, the respondent's argument that the patent contains repeatable examples and extensive disclosure in paragraphs [0027] to [0046] about how to put the invention into effect also fails.

Common general knowledge of the skilled person

32. The respondent during the oral proceedings before the board conceded that the conditions for achieving a fermentation broth comprising subtilisin crystals were not disclosed in the patent in suit. They however maintained that this was not required in view of the common general knowledge of the skilled person.

33. With respect to documents D2, D4 and D7, relied on by the respondent as evidence that fermentation broths comprising subtilisin crystals were well known in the state of the art, the board notes that document D2 discloses that addition of a flocculant or a precipitation agent, such as a salt or a low-molecular-weight organic solvent, to the fermentation broth containing subtilisin causes the protein to precipitate (see page 6, line 34 to page 7, line 3 and Example 3). Document D4 discloses the formation of α -amylase crystals in a fermentation broth (see Example 1), but does not contain any disclosure as regards the formation of subtilisin crystals in a fermentation broth (see column 1, lines 22 to 26 and column 2, lines 4 to 11 and 17 to 19). In document D7, the fermentation broth containing subtilisin is treated with coagulants or flocculants to produce a crystalline suspension (see abstract and Example 1).
34. The board concurs with the appellant that none of documents D2, D4 and D7 discloses a fermentation broth containing subtilisin crystals formed due to high yield. Instead, they provide evidence that additional measures were employed in the state of the art to actively convert the soluble form of subtilisin to crystals and/or precipitate.
35. The respondent further argued, relying on documents D49 and D53, that improvements in fermentation yields were the prevailing situation in the protease fermentation industry at the priority date of the patent.
36. Document D49 was published after the priority date of the patent (see sections I. and III.). Thus the question arises whether or not document D49 can be accepted at all as being an account of the prevailing

situation in the art at the effective date of the patent. In the board's judgement, this question can however be left unanswered since the document in fact does not support the respondent's case. Indeed, document D49 discloses that "*[a]dvances in expression technology have resulted in the ability to obtain relatively high enzyme concentrations (e.g., 10-100 g/l) in fermentation broth. In some cases, the expression level exceeds the solubility limit of an enzyme of interest, and the enzyme is present in a precipitated or crystalline form at the end of fermentation*" (see page 3, paragraph [11]). The document however does not provide any information on these "*advances in expression technology*", i.e. is silent on how they were achieved. Further, with respect to subtilisin, document D49 provides no information on a fermentation broth comprising crystals. On the contrary, it reports a fermentation broth containing soluble subtilisin which requires addition of flocculant to form a precipitate (see Example 7).

37. Document D53 discloses, in the context of manufacturing proteases for detergents, that "*[y]ields could be improved to high levels and, thanks to the high purity of the secreted enzyme, it could even be crystallized by addition of salts*" (emphasis added, see page 253, 4th paragraph).
38. Thus the board considers that neither document D49 nor document D53 provides evidence that improvements in fermentation yield leading to subtilisin concentrations exceeding the solubility limit and resulting in spontaneous crystallisation were widespread in the state of the art.

39. With respect to the respondent's assertion that the fermentation yield could be raised above the solubility limit by optimising the production microorganism using routine techniques that belonged to the common general knowledge of the skilled person, the board notes that no corroborating evidence was provided by the respondent in this respect.
40. To the contrary, it can be inferred from document D53, the sole document relied on by the respondent as representing the common general knowledge of the skilled person, that years of optimisation are required to improve the fermentation yield (see Figure 4). Moreover, yield improvement by the measures studied in document D53, i.e. chemical mutagenesis, medium optimisation and genetic engineering, reach a plateau after seven years of research (see Figure 4), without reportedly leading to the formation of crystals (see page 253, 3rd paragraph).
41. Notably, the respondent had themselves submitted that the "*dramatic*" increases referred to in paragraph [0002] of the patent "*had arisen as a result of years of optimisation and improvement in the production process and production microorganism*". However, evidence that the skilled person was aware of the concrete measures of optimisation and improvement to be taken to reliably reach such high yields in fermentation resulting in subtilisin crystals had not been provided by the respondent.
42. In the board's opinion, a reasonable amount of trial-and-error experimentation may be acceptable to acknowledge that the claimed invention can be carried out without undue burden. This however presupposes that sufficient information is available that leads the

skilled person directly towards success through the evaluation of initial failures. In the present case, and based on the evidence on file, the board considers that information on critical process variables (see points 40. and 41. above) is lacking. The skilled person would therefore need to repeat the lengthy optimisation and improvement process, without any guarantee that fermentation conditions resulting in the formation of subtilisin crystals during fermentation due to high yield would be achieved. Such a situation is often described in the case law as an invitation to perform a "research program" and considered to amount to an undue burden (see CLBA, section II.C.6.7).

43. The respondent's proposition that the skilled person only needed to run the fermentation long enough to reach supersaturation is not found to be persuasive either. The skilled person is well aware that during a fermentation process microorganisms eventually stop producing. The skilled person would thus not expect supersaturation to be able to be reached by merely extending the duration of the fermentation process, and in particular not in the absence of any evidence in the state of the art that supersaturation with respect to subtilisin has ever been reached in a fermentation broth and has led to crystal formation (see point 33. above).

44. The respondent also argued that the skilled person could artificially induce crystal formation by changing the factors known to affect the solubility of enzymes in general, e.g. by modifying the salt concentration and/or pH of the fermentation broth or by spiking the fermentation broth by adding crystals and/or amorphous solid subtilisin.

45. However, the embodiment under consideration (see point 22. above) relates to the presence of subtilisin crystals in a fermentation broth due to high yield, and the purpose of the claimed method is to dissolve these subtilisin crystals so that soluble subtilisin can be separated from the sludge. If the fermentation yield is so low that all of the subtilisin is still in the soluble phase, the skilled person cannot be assumed to even consider artificially inducing the formation of crystals only to dissolve them again in the next step. Instead, the skilled person would remove soluble subtilisin from the sludge immediately by centrifugation or filtration. Furthermore, artificial induction of subtilisin crystal formation is not disclosed or even hinted at in the patent.
46. In view of the above considerations, the board concurs with the appellant that there exists no common general knowledge on how to obtain the high yield of subtilisin needed to achieve a fermentation broth with crystals.

Post-published experimental evidence

47. The opposition division in the decision under appeal accepted that the post-published document D29 demonstrated "*that crystals can be formed and subsequently solubilized in accordance with the examples of the patent*", and the respondent maintained on appeal that document D29 confirmed that subtilisin crystals could be formed in a fermentation broth.
48. Sufficiency of disclosure must, in principle, exist at the effective date of a patent, while post-published documents may be used as evidence that the disclosure is reproducible without undue burden only under certain

circumstances (see also CLBA, section II.C.6.8).

49. The board notes that whether or not in the case at hand, where the patent provides no guidance for achieving a fermentation broth comprising subtilisin crystals, post-published evidence can remedy a lack of sufficiency of disclosure can in fact be left unanswered because, in the board's opinion, the disclosure in document D29 is manifestly unsuitable as evidence that "*crystals can be formed (...) in accordance with the examples of the patent*".

50. In fact, the examples of the patent in suit do not disclose any details as to how a fermentation broth comprising subtilisin crystals is prepared (see points 25. and 26. above), and this was conceded by the respondent (see point 32. above). Document D29 merely states that "[a] *fermentation broth containing subtilisin was prepared according to Example 1 of European patent no 2125865 [the patent]*". No experimental protocol and no information on the bacterial strain used or any of the fermentation parameters such as medium components, temperature, pH, duration are disclosed in document D29.

51. Furthermore, the appellant has provided experimental data showing that fed-batch fermentation using complex fermentation broth of a *Bacillus licheniformis* host cell producing a *Bacillus lentus* subtilisin (Savinase) did not lead to subtilisin crystals (see document D48, Experiment 1). Adding dialysed *Bacillus lentus* subtilisin (Savinase), stirring and storing overnight (see document D48, Experiment 1) and increasing the subtilisin concentration to 90 g/kg by adding lyophilised *Bacillus lentus* subtilisin (Savinase) did not lead to subtilisin crystals either (see

document D48, Experiment 2).

52. Firstly, the board considers that in the circumstances of the present case (see point 30. above) the provision of evidence in the form of experimental data was not even required to discharge the appellant's burden of proof.
53. Secondly, the board finds the respondent's criticisms with respect to these experimental data not to be persuasive. In fact, the very host cell and subtilisin used in Experiment 1 of document D48 are those mentioned in the examples of the patent, the disclosed fermentation process is the preferred one according to paragraph [0025] of the patent, the medium is one of the media disclosed in paragraph [0021] of the patent and the patent states that the "*microorganism producing the protease of interest may be fermented by any method known in the art*" (see paragraph [0021]). Furthermore, the board points out that providing high yield is the sole measure disclosed in the patent for generating subtilisin crystals, although the fermentation conditions leading to high yields and to subtilisin crystals are not disclosed (see point 30. above). The board notes further that, in the experiments reported in document D48, very high yields of subtilisin were simulated by supplementing the fermentation broth with subtilisin, but no crystallisation was observed.
54. The board is thus satisfied that document D48 provides evidence that the appellant, despite making a reasonable effort, was unable to put the claimed invention into practice.

Conclusion on sufficiency of disclosure (Article 100(b) EPC) - main request

55. The board concludes from the above considerations that the appellant has discharged their burden of proof and that the respondent has not established that the embodiment under consideration was indeed workable for the skilled person without undue burden using common general knowledge at the effective date of the patent.

56. The ground for opposition under Article 100(b) EPC thus prejudices the maintenance of the patent as granted.

Auxiliary requests 1, 2, 3, 5, 6 and 7 - claim 1

Sufficiency of disclosure (Article 83 EPC)

57. The above reasoning and conclusion for claim 1 of the main request apply, *mutatis mutandis*, to the same claim of these requests. This was not disputed by the respondent.

Auxiliary requests 4, 8 and 9 - claim 1

Sufficiency of disclosure (Article 83 EPC)

58. The claim has been limited to subtilisin crystals and/or subtilisin precipitate obtained from a *Bacillus* cell.

59. As for claim 1 of the main request (see point 22. above), the claim is understood to relate, in one embodiment, to diluting a fermentation broth that comprises subtilisin crystals as a consequence of high fermentation yields.

60. The respondent's argument that the claimed subject-matter was closer to the examples and could therefore be carried out by the skilled person without undue burden is not found to be persuasive. The examples of the application provide no guidance with respect to the technical measures to be applied in the fermentation process of subtilisin proteases to achieve such high yields that subtilisin crystallises spontaneously in the fermentation broth during fermentation, see also points 25. to 30. above.

61. Therefore the above reasoning and conclusion for claim 1 of the main request apply, *mutatis mutandis*, to this claim of these requests.

Conclusion on sufficiency of disclosure (Article 83 EPC) - auxiliary requests 1 to 9

62. The claimed invention is not disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art as required by Article 83 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The patent is revoked.

The Registrar:

The Chair:



A. Chavinier Tomsic

B. Claes

Decision electronically authenticated