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**Datasheet for the decision
of 7 December 2006**

Case Number: T 0886/02 - 3.3.08

Application Number: 93914652.8

Publication Number: 0651794

IPC: C12N 9/28

Language of the proceedings: EN

Title of invention:

Mutant α -amylase, detergent, dish washing agent, and
liquefaction agent

Applicant:

Novozymes A/S

Opponent:

-

Headword:

Mutant alpha-amylase/NOVOZYMES

Relevant legal provisions:

EPC Art. 54, 56, 83, 84, 87, 123(2)

Keyword:

"Amended claim request - added matter (no)"
"Clarity (yes)"
"First priority date validly claimed (yes)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:

G 0002/98, T 0195/84

Catchword:

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Case Number: T 0886/02 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 7 December 2006

Appellant: Novozymes A/S
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Representative: Stevens, Ian Edward
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Decision under appeal: Decision of the Examining Division of the
European Patent Office posted 16 April 2002
refusing European application No. 93914652.8
pursuant to Article 97(1) EPC.

Composition of the Board:

Chairman: F. Davison-Brunel
Members: M. R. Vega Laso
T. Karamanli

Summary of Facts and Submissions

- I. European Patent Application No. 93 914 652.8 with the title "Mutant α -amylase, detergent, dish washing agent, and liquefaction agent" was filed as International application under the PCT (PCT/DK93/00230) on 6 July 1993, and published under the International publication No. WO 94/02597. The application claimed the priority of three earlier Danish patent applications of 23 July 1992, 16 December 1992 and 15 March 1993, respectively.
- II. In a decision posted on 16 April 2002, the application was refused by the examining division under Article 97(1) EPC. In the reasons for the refusal, the examining division held that the subject-matter of claim 1 then on file did not enjoy the priority of the earlier Danish applications of 23 July 1992 and 16 December 1992, and, consequently, document (3) (see *infra*), which validly claimed the priority of an earlier US application filed on 11 February 1993, was comprised in the state of the art under Article 54(3) and (4) EPC. Since the disclosure of document (3) anticipated the subject-matter of at least claim 1, the sole claim request then on file was considered not to fulfil the requirement of novelty (Article 54 EPC).
- III. The appellant (applicant) filed a notice of appeal against this decision and paid the appeal fee. With the statement setting out the grounds of appeal, the appellant submitted as its main request a set of claims identical to the claim request on the basis of which the application had been refused, as well as four new auxiliary requests. In the event that any of these requests, while being considered to meet the

requirement of Article 54 EPC, was however found not to fulfil one or more of the further requirements of the EPC, remittal of the case to the examining division was requested. As a further subsidiary request, the appellant requested oral proceedings under Article 116 EPC.

IV. The examining division did not rectify its decision and the case was remitted to the board of appeal (Article 109 EPC).

V. The appellant was summoned to oral proceedings. In a communication under Article 11(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed to the summons, the board expressed its preliminary opinion on the issues of priority and novelty, and pointed to several formal deficiencies in the claims, in particular concerning various disclaimers which had been introduced in claim 1 of the second auxiliary request.

VI. In response to this communication, the appellant filed on 6 November 2006 a new main request and four auxiliary requests which replaced the claim requests previously on file.

VII. Oral proceedings were held on 7 December 2006. At the outset of the proceedings, the board, after hearing the appellant, decided not to admit into the proceedings the main claim request (which had been filed as first auxiliary request on 6 November 2006). The issue of priority was then discussed for the first auxiliary request (filed as main request on 6 November 2006). Following this discussion, the appellant submitted an

amended claim request (claims 1 to 4) which, after withdrawal of all further claim requests on file, became the sole claim request. Moreover, the request for remittal of the case to the examining division was withdrawn.

VIII. Independent claim 1 of the amended claim request reads:

"1. Mutant *Bacillus licheniformis*, *Bacillus amyloliquefaciens* or *Bacillus stearothermophilus* alpha-amylase characterised in that the methionine amino acid residue in position 197 in *Bacillus licheniformis* or *Bacillus amyloliquefaciens* alpha-amylase or in position 200 in *Bacillus stearothermophilus* alpha-amylase is exchanged with a Leu, Ile, Asn, Ser, Gln, Asp or Glu."

Dependent claim 2 was directed to a particular embodiment of the mutant alpha-amylase of claim 1, in which the methionine amino acid residue was exchanged with Leu. Independent claims 3 and 4 related to, respectively, a detergent and a dish washing agent comprising a mutant alpha-amylase as claimed.

IX. The following documents are mentioned in the present decision:

P1: Priority document of the application corresponding to the Danish patent application 0946/92, filed on 23 July 1992;

(1): D.A. Estell et al., The Journal of Biological Chemistry, 10 June 1985, Vol. 260, No. 11, pages 6518 to 6521;

(2): WO 91/16423, published on 31 October 1991;

(3): WO 94/18314, published on 18 August 1994.

X. The arguments put forward by the appellant, as far as they are relevant for this decision, may be summarized as follows:

Basis for the amended claim 1 was found on page 3, lines 11, 17 and 19 of the application as filed. The amended claims were clear and concise, and were supported by the description as a whole. Thus, the formal requirements of the EPC were met.

Since the same subject-matter as in claim 1 was disclosed in the first priority application, this claim enjoyed the priority date of 23 July 1992. Hence, contrary to the finding in the decision under appeal document (3) was not comprised in the state of the art under Article 54(3) and (4) EPC. The subject-matter of claim 1 was thus novel.

Document (3) was published after the filing date of the present application and, consequently, was not comprised in the state of the art relevant to the assessment of inventive step. None of the prior art documents on file contained any teaching that linked the methionine residue at position 197 in the *B. licheniformis* or *B. amyloliquefaciens* alpha-amylase, or the homologous methionine residue at position 200 in the *B. stearothermophilus* alpha-amylase to the stability of the enzyme in an oxidative environment. Accordingly, it was not obvious to the person skilled in the art that an exchange of those methionine

residues might have an effect on oxidation stability. Therefore, the claimed mutant alpha-amylases involved an inventive step within the meaning of Article 56 EPC.

- XI. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of claims 1-4 filed during oral proceedings.

Reasons for the Decision

Admission of the amended request into the proceedings

1. The amended claim request now for consideration differs from the claim request underlying the decision under appeal essentially in that claim 1 has been restricted to mutant alpha-amylases derived from three specific *Bacillus* alpha-amylases, in which a particular methionine residue (M197 in *B. licheniformis* or *B. amyloliquefaciens* alpha-amylase, and M200 in *B. stearothermophilus* alpha-amylase) is exchanged with a Leu, Ile, Asn, Ser, Gln, Asp or Glu residue. In the board's view, the amended claim request represents a serious attempt at overcoming objections raised by the board either in its communication under Article 11(1) RPBA or during the oral proceedings. Therefore, the claim request was admitted into the proceedings.

Articles 123(2), 84 and 83 EPC

2. Basis for the subject-matter of the amended claim 1 is found on page 1, lines 23 to 28 of the application as filed (mutant alpha-amylase characterized in that a

methionine residue is exchanged with Leu, Ile, Asn, Ser, Gln, Asp or Glu), read in combination with the statements on page 2, lines 28 to 31 (methionine residue in position 197 in *B. licheniformis* or methionine in homologous positions in other alpha-amylases) and page 3, lines 10 to 12 (methionine in positions 200 and 197 as homologous positions in, respectively, *B. stearothermophilus* and *B. amyloliquefaciens* alpha-amylases). The same passages of the application as filed serve as basis for the subject-matter of the dependent claim 2, which is directed to one of the alternative embodiments encompassed by claim 1. A detergent and a dish washing agent comprising a mutant alpha-amylase as claimed (claims 3 and 4) are disclosed on page 3, lines 24 to 25, and page 6, lines 13 to 15 of the application as filed. Thus, the amendments to the claims do not introduce subject-matter which extends beyond the content of the application as filed.

3. Moreover, in the board's judgement the amended claims are clear and supported by the description.
4. Thus, the requirements of Articles 123(2) and 84 EPC are fulfilled.

Priority (Article 87 EPC) and relevant state of the art

5. In view of the findings in the decision under appeal (see Section II above), the issue of priority must be examined anew in respect of the subject-matter of the amended claim request.

6. Mutant alpha-amylases derived from the *B. licheniformis*, *B. amyloliquefaciens*, or *B. stearothermophilus* alpha-amylases are disclosed on page 1, lines 24 to 27 of the first priority document (P1), these mutant alpha-amylases being characterized by the fact that one methionine residue is exchanged with a Leu, Ile, Asn, Ser, Gln, Asp or Glu residue (see page 1, lines 17 to 20). Furthermore, the methionine residue at position 197 in the *B. licheniformis* or *B. amyloliquefaciens* alpha-amylases and the methionine residue at position 200 in the *B. stearothermophilus* alpha-amylase are mentioned on page 2, lines 12 to 22 of P1 among other possible methionine residues to be exchanged. A detergent and a dish washing agent comprising a mutant alpha-amylase according to the invention are disclosed on page 2, lines 6 to 11 of P1.
7. In view of these passages, the board judges that the subject-matter of the amended claims 1 to 4 is directly and unambiguously derivable from the first priority application (P1) and that, therefore, the requirement for claiming priority of the "same invention" referred to in Article 87(1) EPC is fulfilled (G 2/98; OJ EPO 2001, 413). Thus, in respect to the amended claims 1 to 4 the claimed priority date of the first priority application (23 July 1992) counts as the date of filing of the present application for the purposes of Article 54(3) EPC (cf. Article 89 EPC).
8. Consequently, the content of the European patent application No. 94 909 609 (published as WO 94/18314; cf. document (3) *supra*) with filing date of 10 February 1994 and claiming the priority of an earlier US application of 11 February 1993, cannot be considered

as comprised in the state of the art under Article 54(3) and (4) EPC relevant to the assessment of novelty.

Novelty, industrial applicability and sufficiency of disclosure (Articles 54(1), 57 and 83 EPC)

9. There are no prior art documents on file describing mutant alpha-amylases with the features specified in claims 1 and 2. Nor are a detergent or a dish washing agent containing a mutant alpha-amylase as claimed disclosed. Novelty is, thus, acknowledged.
10. Industrial applicability is to be seen in the field of additives for detergents and dish washing agents containing oxidizing agents. The improved stability of the claimed alpha-amylases in the presence of oxidizing agents is disclosed in the examples of the application (see Tables 1 and 2). The requirements of Article 57 EPC are fulfilled.
11. A method for obtaining mutants by site-directed mutagenesis as well as the amino acid sequence of the *B. licheniformis*, *B. amyloliquefaciens* and *B. stearothermophilus* alpha-amylases are described in the application as filed by reference to several scientific publications (see page 7, lines 1 to 15 of the application as filed). In the absence of evidence to the contrary, the board considers that the requirements of Article 83 EPC are fulfilled.

Inventive step (Article 56 EPC)

12. The closest prior art is document (2) which teaches the production of chemically modified detergent enzymes, in

particular alpha-amylases, by exchange of one or more methionines in the enzyme with cysteines, and subsequent chemical modification of the cysteines in order to substitute the hydrogen of the HS-group into a group of the general formula R^1S- , wherein R^1 is C_{1-6} -alkyl. The aim of the work is to solve the problem of low oxidation stability affecting detergent enzymes used in an oxidative environment, eg in the presence of bleach active ingredients. Document (2) further teaches a detergent composition comprising an oxidation stable detergent enzyme as described.

13. Starting from the closest prior art, the technical problem to be solved can be defined as producing further mutant alpha-amylases with improved oxidation stability.
14. Neither document (2) nor any of the further prior art documents on file (which, in any case, are not concerned with alpha-amylases) suggest the possibility of obtaining further mutant alpha-amylases with improved oxidation stability. However, taking into account that furthering the existing state of knowledge belongs to the routine tasks with which the skilled person is constantly occupied (see, for example, T 195/84, OJ EPO 1986, 121), the formulation of this technical problem is considered obvious.
15. The proposed solution to the technical problem formulated above is a mutant alpha-amylase as defined in claim 1. In view of the experimental results provided in the application (see Tables 1 and 2), the board is convinced that the technical problem is in fact solved by mutant *B. licheniformis* alpha-amylases

in which the methionine residue at position 197 has been exchanged with a Leu, Ile, Asn, Ser, Gln or Asp residue. Moreover, even though no experimental results are provided in the application for mutant *B. amyloliquefaciens* or *B. stearothermophilus* alpha-amylases as claimed, having regard to the structural similarity of these alpha-amylase proteins with the alpha-amylase of *B. licheniformis* - as described on, for example, page 3, lines 2 to 12 of the application as filed -, it can reasonably be expected that the substitution of a methionine corresponding to M197 in *B. licheniformis* (ie M197 in *B. amyloliquefaciens* alpha-amylase and M200 in *B. stearothermophilus* alpha-amylase) by any of the residues Leu, Ile, Asn, Ser, Gln or Asp will produce the same technical effect, ie an improvement of the oxidation stability of the alpha-amylase.

16. With respect to the claimed mutant alpha-amylases having a Glu residue instead of methionine, the application as filed provides no experimental support for the alleged effect. However, it seems plausible that the exchange of a readily oxidizable amino acid residue (methionine) with a non-oxidizable amino acid residue (glutamic acid) at a position of the alpha-amylase which, in view of the results obtained for the *B. licheniformis* alpha-amylase, appears to be important for the stability of the enzyme in an oxidative environment, may result in a mutant alpha-amylase with improved oxidation stability. Thus, in the absence of any evidence to the contrary the board acknowledges that the technical problem has been solved over the whole scope of claim 1.

17. Hence, the sole issue that remains to be decided in connection with inventive step is whether or not, starting from document (2) and having regard to the information provided in further prior art documents on file, the subject-matter of claim 1 was obvious. In this respect, the board notes that, even if at the priority date it was known that the oxidative stability of subtilisin (a protease) can be improved by replacement of an oxidatively sensitive residue which is critical for activity (methionine 222) by a Ser, Ala or Leu residue (see document (1), abstract), neither this document nor any of the further prior art documents on file gives the skilled person any pointers on the methionine residue at position 197 in the *B. licheniformis* or *B. amyloliquefaciens* alpha-amylase, or the homologous methionine residue at position 200 in the *B. stearothermophilus* alpha-amylase, as a putative target for exchange with a different amino acid residue, in particular Leu, Ile, Asn, Ser, Gln, Asp or Glu, with the aim at improving the oxidation stability of the enzyme.

18. Thus, it is concluded that, having regard to the state of the art as reflected in the prior art documents on file, the invention defined in claim 1 provides a hitherto unforeseeable possibility of improving the oxidation stability of various *Bacillus* alpha-amylases. Therefore, inventive step is acknowledged in respect of the subject-matter of claim 1.

19. Independent claims 3 and 4 relate to, respectively, a detergent and a dish washing agent comprising a mutant alpha-amylase with the features specified in claim 1.

Their subject-matter involves an inventive step for the same reasons as given in relation to that of claim 1.

Amendments to the description

20. The board is satisfied that the amendments introduced into the application in order to adapt the description to the amended claims do not offend against Article 123(2) EPC.

21. Summarising the above: the amended claims 1 to 4 and the description adapted thereto filed at the oral proceedings fulfil the requirements of the EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to grant a patent in the following version:

Description: pages 1-3 and 6-8 received during oral proceedings and pages 4, 5 and 9-14 as originally filed.

Claims: No. 1-4 received during oral proceedings.

The Registrar:

The Chairman:

A. Wolinski

F. Davison-Brunel