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**Datasheet for the decision  
of 18 December 2007**

**Case Number:** T 1521/06 - 3.3.08

**Application Number:** 94915270.6

**Publication Number:** 0670367

**IPC:** C12N 9/28

**Language of the proceedings:** EN

**Title of invention:**

Liquefying alkaline alpha-amylase, process for producing the same, and detergent composition containing the same

**Patentee:**

KAO CORPORATION

**Opponents:**

NOVOZYMES A/S  
HENKEL KGaA

**Headword:**

Amylase/KAO

**Relevant legal provisions:**

EPC Art. 54, 56, 83  
EPC R. 31

**Keyword:**

"Main request: novelty (yes)"  
"Inventive step (yes)"  
"Sufficiency of disclosure (yes)"

**Decisions cited:**

-

**Catchword:**

-



Case Number: T 1521/06 - 3.3.08

**D E C I S I O N**  
of the Technical Board of Appeal 3.3.08  
of 18 December 2007

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**Decision under appeal:** Interlocutory decision of the Opposition  
Division of the European Patent Office posted  
26 July 2006 concerning maintenance of European  
patent No. 0670367 in amended form.

**Composition of the Board:**

**Chairman:** L. Galligani  
**Members:** T. J. H. Mennessier  
C. Rennie-Smith

## Summary of Facts and Submissions

- I. The patentee (appellant I) and the two opponents (opponent 01/appellant II and opponent 02/appellant III) lodged appeals against the interlocutory decision of the opposition division dated 26 July 2006, whereby European patent No. 0 670 367, which had been granted on European application No. 94 915 270.6 originating from the international publication WO 94/26881, was maintained in an amended form on the basis of the auxiliary request filed at the oral proceedings held on 31 January 2006. The main request then on file had been refused for non-compliance with the requirements of Article 83 EPC.
- II. The main request consisted of claims 1 to 8 as granted.

Claim 1 read as follows:

"1. A liquefying alkaline  $\alpha$ -amylase having the following enzymatic properties:

- a) hydrolyzing 1,4- $\alpha$ -glucosidic linkages in starches, amylose, amylopectin and partial degradation products thereof and forms glucose (G1), maltose (G2), maltotriose (G3), maltotetrose (G4), maltopentose (G5) and maltohexose (G6) from amylose;
- b) not acting on pullulan;
- c) having an isoelectric point of 8.7 to 9.7;
- d) having an optimum pH of from 8.0 to 10

which is derived from the bacterium belonging to the genus Bacillus and which has a sequence of

Asn-Gly-Thr-Met-(Met)-Gln-Tyr-Phe-Glu-Trp in its N-terminal amino acid region."

Claim 2 was dependent on claim 1 and read:

"2. A liquefying alkaline  $\alpha$ -amylase according to claim 1, further **characterized by**

- a) acting in a pH range of from 5.0 to 11.0,
- b) having an optimum pH of from 8.0 to 9.0;
- c) being stable in a pH range of from 6.5 to 10.0;
- d) retaining at least 50% of its activity in a pH range of from 5.0 to 10.5 after treated at 40°C for 30 minutes;
- e) acting in a temperature range of from 20°C to 80°C, with the optimum temperature being 45°C to 55°C;
- f) being stable at temperatures of 50°C or lower when treated for 30 minutes in a glycine-salt-sodium hydroxide buffer having pH 8.5;
- g) having a molecular weight of 50,000  $\pm$  5,000 as measured in accordance with the sodium dodecyl sulfate polyacrylamide gel electrophoresis;
- h) having an isoelectric point of around pH 9.2 when measured by isoelectric focusing electrophoresis;
- i) is extremely stable against K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> Mn<sup>2+</sup>, Ba<sup>2+</sup>, Fe<sup>2+</sup>, Fe<sup>3+</sup> and Al<sup>3+</sup>;
- j) being substantially free from activity inhibition by surfactants such as sodium linear alkylbenzene sulfonates, sodium alkylsulfate esters, sodium polyoxyethylene alkylsulfate esters, sodium alkylsulfonates, soaps or polyoxyethylene alkyl ethers."

Claim 3 was directed to a process for the preparation of a liquefying alkaline  $\alpha$ -amylase according to any one of claims 1 to 2. Claim 4 was dependent on claim 3 and was directed to a particular embodiment thereof.

Claim 5 was directed to a detergent composition comprising the liquefying alkaline  $\alpha$ -amylase according to any one of claims 1 to 2. Claims 6 to 8 were dependent on claim 5 and were directed to particular embodiments thereof.

- III. The patent had been opposed on the grounds as set forth in Articles 100(a) and (b) EPC that (i) the invention was neither new nor inventive (Articles 54 and 56 EPC) and (ii) the invention was not sufficiently disclosed (Article 83 EPC).
- IV. The statements setting out the grounds of appeal were filed. Appellant I's statement was accompanied by three auxiliary requests.
- V. Both appellants II and III replied to the appellant I's statement of grounds of appeal with letters of 20 and 12 April 2007, respectively.
- VI. In reply to the statements of grounds of appeal of appellants II and III, appellant I filed additional submissions with a letter of 23 April 2007 which were accompanied by a fourth auxiliary request.
- VII. A communication under Article 11(1) (now Article 15(1) - see OJ EPO 2007, 543) of the Rules of Procedure of the Boards of Appeal presenting some preliminary and

non-binding views of the Board was sent to the parties on 20 July 2007.

VIII. Appellant II filed additional submissions with a letter dated 12 November 2007.

IX. With a letter dated 15 November 2007, appellant I filed further submissions which were accompanied by three auxiliary requests, designated I to III, to replace all the auxiliary requests on file.

X. Oral proceedings took place on 18 December 2007.

XI. The following documents are referred to in the present decision:

(D1) WO 95/26397 (published on 5 October 1995; international filing date: 29 March 1995; earliest priority date: 29 March 1994)

(D5) esp@cenet database (<http://v3.espacenet.com>); print of 30 April 2004 - abstract for patent application JP-A-4058885 published on 25 February 1992

(D6) US-A-5,147,796 (published on 15 September 1992)

(D8) Akira Tsukamoto et al., Biochemical and Biophysical Research Communications, Vol. 151, No. 1, 29 February 1998, Pages 25 to 31

(D9) WO 91/00345 (published on 10 January 1991)

(D10) EP-A1-0 418 835 (published on 27 March 1991)

- (D11) EP-A2-0 450 627 (published on 9 October 1991)
  
- (D16) "Handbook of Amylases and Related Enzymes - Their Sources, Isolation Methods, Properties and Applications", Edited by the Amylase Research Society of Japan, Pergamon Press, 1988, Pages 40 to 45
  
- (D28) "Microbiology of Extreme Environments and its Potential for Biotechnology", Edited by M. S. Da Costa, J. C. Duarte and R. A. D. Williams, Elsevier Applied Science, London and New York, Proceedings of the Federation of European Microbiological Societies Symposium held in Troia, Portugal, 18 to 23 September 1988
  
- (D34) Scientific report enclosed with appellant II's letter of 5 December 2006
  
- (D36) Declaration of Henrik Østdal dated 9 November 2007
  
- (D37) Declaration of Carsten Andersen dated 8 November 2007

XII. The submissions made by appellant I, insofar as they are relevant to the present decision, may be summarised as follows:

Main request

*Novelty (Article 54 EPC)*

The opposition division decided that the priority of the patent was not valid for the reason that the claimed isoelectric point (pI) range of "8.7 to 9.7" was considered not to be directly and unambiguously derived from the value of "about 9.2" in the priority document. As far as the pI was concerned the only difference was the error range of "0,5" which had been included in the patent but not in the priority, where the value without the error range was disclosed. The priority was validly claimed because the mere inclusion of the error margin did not change the character of the invention which remained the same.

There was no evidence whatsoever submitted by appellant II and/or appellant III to demonstrate that the enzyme in document D1 did not act on pullulan. Document D1 was a patent application of appellant II, which thus should have been able to perform experiments with this enzyme with regard to its activity on pullulan, but had failed to do so. Therefore, claim 1 was new over document D1.

The activity of the enzyme of document D8 on pullulan was measured in the experiments submitted by appellant II (see document D34). It was clearly stated therein that "*The SP707 was shown to have 0.1 to 0.2% activity towards pullulan compared to either amylose or amylopectin.*". The conclusions of the experimental report were that: "*SP 707 had only very minute activities on pullulan as determined using commercial pullulan.*". Thus, the enzyme was shown to have some



activity on pullulan. While appellant II attempted to explain the activity of the SP707 enzyme, it completely failed to provide any experimental data that would confirm that this activity was not genuine. Thus, claim 1 was new over document D8.

In the experiments submitted by appellant II, the pI as measured for the enzyme of the invention was stated to range between 8.4 to 8.66 based on the gel run in the experimental report. This estimated pI did not correspond to the pI as reported in the patent. Thus, it could only be concluded that something was not correct in the experiments provided by appellant II. It was questionable whether the experimental comparisons reported in document D34 were all performed with the same enzyme as the one exemplified in the patent. The presence of two bands on the gel, whereas only one band would have been expected (a particular enzyme might have only one pI), was a clear indication that the experiments had been performed in an inappropriate manner, possibly leading to an artefact. Thus, the scientific report submitted by appellant II (see document D34) did not qualify for a novelty assessment and could not be taken as a reliable indicator of the pI value of the enzyme of document D8. Thus, it had not been established by appellant II that the enzyme of document D8 had a pI value falling within the range indicated in claim 1. Also for this reason claim 1 was new over document D8.

*Inventive step (Article 56 EPC)*

Document D5 represented the closest state of the art. When starting from this document, the skilled person

would have had to alter the Bacillus amylase it described so as to shift the pI from the reported 4,3 to the range given in claim 1 of 8,7 to 9,7. Document D5 did not motivate the skilled person to do this nor describe how to achieve it. Nothing in the proceedings demonstrated that the skilled person could have arrived at an enzyme according to claim 1.

Even if one were to consider document D8 as the closest state of the art, there was no indication whatsoever from this document that the DNA sequence disclosed therein could be used to screen Bacillus strains for a gene encoding a liquefying alkaline  $\alpha$ -amylase having the properties of the enzyme of claim 1. Whereas the technical problem solved by the invention was the provision of an  $\alpha$ -amylase without pullulanase activity that had improved qualities for use in a detergent and was easy to purify, documents D9, D10 and D11 would have taught away from the invention as they disclosed either a subtilisin protease (document D9) or an  $\alpha$ -amylase with pullulanase activity (documents D10 and D11).

*Sufficiency of disclosure (Article 83 EPC)*

The inventors performed an extensive search of naturally occurring microorganisms in general to find a liquefying alkaline  $\alpha$ -amylase. As a result, they unexpectedly found that an enzyme having the properties of the liquefying  $\alpha$ -amylase of claim 1 could be obtained from Bacillus. This was exemplified using the deposited Bacillus sp. KSM-AP1378 (FERM BP-3048). Once this was disclosed, it would have been routine experimentation to find additional enzymes in Bacillus

having the properties of the enzyme of claim 1. In order to do so, one was provided with all the assays needed to screen for such bacteria and determine whether they contained a liquefying alkaline  $\alpha$ -amylase that had the measurable functional and structural features of claim 1.

There could be no doubts that the specific Bacillus sp. KSM (FERM BP-3048) could be grown and that the enzyme obtained therefrom could be used to repeat the teaching in the patent.

As far as the screening method for enzymes from other Bacillus strains was concerned, paragraph 0015 in the patent (see page 4) explicitly stated that the invention was not particularly limited to any microorganism. Thus, in the context of the granted claims, the skilled person would have readily understood that the invention was not limited to any particular Bacillus. The state of the art contained no evidence that an  $\alpha$ -amylase with the claimed features could not be obtained from a Bacillus strain other than the specified one.

The assay to determine whether an enzyme had the amylase activity referred to in claim 1 was described in paragraphs 0019 and 0020 of the patent (see page 5). The skilled person would have also performed this assay using the substrates, including pullulan, listed in Table 1 (see page 5 of the patent).

All the described assays, including the assay for the pullulanase activity, were performed with the same concentration of substrates and the relative activity

of the enzyme on these various substrates was reported to a decimal place after the zero. Thus, the skilled person was also provided with an explicit teaching as to the precision of these measurements. In Table 1 it was explicitly stated that at a concentration of 0,25% the relative activity of the enzyme on pullulan was 0,0% as compared to the relative activity of the enzyme on soluble potato starch of 100,0% at a concentration of 0,25%.

Thus, the skilled person was provided with a clear and complete teaching in paragraphs 0019 to 0022 as to how to assess whether any given enzyme obtained from a Bacillus strain was an  $\alpha$ -amylase or not, was liquefying or not, and acted on pullulan or not within the meaning of claim 1.

The skilled person was certainly in a position to measure the pH optimum of an enzyme in the assay described in paragraph 0020 with the buffers given in paragraph 0019 at a concentration of 40 mM to determine the optimum pH. Thus, he/she was provided with a clear and complete teaching as to how to assess whether any given enzyme was an alkaline  $\alpha$ -amylase having an optimum pH of from 8,0 to 10,0 within the meaning of claim 1.

The specification explicitly taught the skilled person to determine the isoelectric point by the commonly known technique of isoelectric focusing electrophoresis. An enzyme having a pI of 8,7 to 9,7 was considered to be an enzyme according to claim 1.

The specification also made clear that the enzymes of the invention had the explicit amino acid sequence in their N-terminal amino acid region as given in claim 1. It was more than clear to the skilled person that the notation of methionine in parenthesis meant that this methionine was optional at this position in the amino acid sequence.

Thus, the patent contained sufficient information for the skilled person to reproduce the invention over its entire scope and also to determine whether a particular enzyme that was derived from a Bacillus strain fell within the scope of claims 1 and 2.

The breadth of claim 1 was commensurate with the contribution to the art of the invention. The existence of an alkaline liquefying  $\alpha$ -amylase which had a high pI was not known in the art. Appellant I had found such an enzyme for the first time and had demonstrated that it was useful in the preparation of detergents. It thus deserved protection for having made this finding. Any third party would be using the teaching of the patent that such an enzyme existed in Bacillus to obtain a further enzyme from Bacillus. In particular, the skilled person starting from Bacillus sp. KSM-AP1378 could obtain by random mutagenesis mutated strains capable of producing amylases encompassed by claim 1.

XIII. The submissions made by appellant II, insofar as they are relevant to the present decision, may be summarised as follows:

Main request

*Novelty (Article 54 EPC)*

Document D1 disclosed an alkaline  $\alpha$ -amylase derived from the Bacillus strain NCIB 12289. The enzyme comprised the N-terminal sequence as referred to in claim 1 which according to paragraph 0032 of the patent (see page 7) was specific to liquefying  $\alpha$ -amylases. Being an  $\alpha$ -amylase it did not act on pullulan. It had inherently the feature b) of claim 1 because hydrolysis of 1,4-alpha-glucosidic linkages to give G1 to G6 products was an inherent feature of  $\alpha$ -amylases. It had a pI of about 8,8 to 9,0 and a pH optimum of 7,5 to 8,5. Therefore, all the features of claim 1 were disclosed in document D1. Thus, as document D1 was entitled to its priority date and the subject-matter at issue was not, claim 1 lacked novelty.

Document D8 disclosed a liquefying alkaline  $\alpha$ -amylase, as indicated in the very last sentence of page 31. Being a liquefying amylase it should produce G1 to G6 since liquefying  $\alpha$ -amylases were known for producing these products from starch (see document D16, page 41). It was not specifically disclosed that it did not act on pullulan. However, this feature was an intrinsic property of all  $\alpha$ -amylases.

The absence of pullulanase activity was confirmed in the declaration of Henrik Østdal (see document D36), in which it was concluded that the very minute activities on pullulan as determined using commercial pullulan found for the amylase of document D8, which were reported in document D34, were not specific towards

pullulan. The apparent activity found in document D34 was not due to the  $\alpha$ -amylase but was an artefact of the method that was used, possibly caused by a starch impurity in the pullulan.

The same scientific report (document D34) also concluded that the amylase of document D8 had a pI between 8,7 and 9,0 and a pH optimum around 9.0. These values were average values obtained after having performed the experiments in triplicate.

Thus, document D8 disclosed an amylase having all the features of an amylase according to claim 1. Therefore, claim 1 lacked novelty over document D8.

*Inventive step (Article 56 EPC)*

Document D8 represented the closest state of the art. It would have been easy to find an alternative to the enzyme of document D8 by random mutagenesing the Bacillus sp. #707 strain described therein with a view to finding an amylase having an alkaline isoelectric point as furthermore suggested by document D9.

*Sufficiency of disclosure (Article 83 EPC)*

The patent did not disclose how the absence of activity on pullulan, a feature of claim 1, could be determined. Further, the patent only disclosed an  $\alpha$ -amylase from one Bacillus strain (KSM-AP1378) without a teaching that would enable the skilled person to obtain other liquefying alkaline  $\alpha$ -amylases within the scope of claim 1. Thus, the disclosure was limited to one strain and it would have required an extensive screening

program in order to provide other alkaline  $\alpha$ -amylases from other microorganisms. The N-terminal sequence as referred to in claim 1 was common to liquefying  $\alpha$ -amylases. Therefore, that feature could not help a skilled person in identifying other amylases according to claim 1. The patent did not describe how the Bacillus strain KSM-AP1378 was found. In the absence of disclosure of any screening method, it was not possible without undue burden to find another Bacillus strain capable of producing such an amylase.

Other technical features of claim 1 (see features b), c) and d)) could not help the skilled person in identifying amylases encompassed thereby other than the specific amylase exemplified in the patent. As regards feature b) of claim 1, no test was described in the patent to assess pullulanase activity. Paragraph 0022 (see page 5) was insufficient in this respect and, moreover, reflected only one particular experiment. The patent failed to disclose how to determine accurately the isoelectric point of an amylase according to claim 1 (see feature c)). Furthermore, there were no indications of the conditions (temperature, etc...) at which the optimum pH of feature d) of claim 1 was to be measured.

Further, the description failed to provide any guidance for the skilled person to determine if a given liquefying  $\alpha$ -amylase was comprised within the scope of claim 2. The reason therefor being that features c), f) and i) of said claim had not been defined in the specification nor did these features have any generally recognised meaning.



Regarding features c) and f) (stability in a pH range of from 6,5 to 10,0, and stability at temperatures of 50°C or lower when treated for 30 minutes in a glycine-salt-sodium hydroxide buffer having pH 8,5, respectively), the description did not disclose how high a residual activity was required in order to qualify the enzyme as being stable. Furthermore, regarding feature c), the length of the residence time at the indicated pH before the residual activity was not determined.

Regarding feature i) (extreme stability against a series of cations), the description did not disclose how high an activity was required in order to qualify the enzyme as being extreme stable. In Table 2 (see page 6 of the patent), the unit of the metal salt concentrations was not given.

- XIV. The submissions made by appellant III, insofar as they are relevant to the present decision, were essentially the same as those made by appellant II. Additional comments were made which can be summarized as follows:

Main request

*Novelty (Article 54 EPC)*

Appellant III essentially agreed with the comments made by appellant II.

*Inventive step (Article 56 EPC)*

Each of documents D6, D10 and D11, which disclosed alternative alkaline enzymes, would have prompted the

skilled person who was aware of the teaching of document D8 (closest state of the art) to look for a liquefying  $\alpha$ -amylase having the features of an amylase according to claim 1.

*Sufficiency of disclosure (Article 83 EPC)*

Only one concrete liquefying  $\alpha$ -amylase produced by a particular Bacillus strain had been disclosed in the patent. That amylase was an exception. It could not be expected that other amylases having an isoelectric point higher than 8,5 could be obtained from other Bacillus strains. The patent did not open a new technical field and the description did not provide the necessary guidance to retrieve further amylases according to claim 1.

Moreover, with the non-limiting expression "derived from" as used therein, claim 1 encompassed amylases, in particular genetically engineered amylases, which were not disclosed in the patent.

Due to the parentheses used in the particular amino acid sequence as referred to therein, claim 1 encompassed amylases lacking a methionine residue which were not disclosed at all in the patent.

- XV. Appellants I (patentee) requested that the decision under appeal be set aside and the patent be maintained as granted or, in the alternative, on the basis of one of auxiliary requests I to III filed on 15 November 2007.

XVI. Appellants II and III (opponents 01 and 02) requested that the decision under appeal be set aside and the patent be revoked.

## **Reasons for the Decision**

### Main request

#### *Novelty (Article 54 EPC)*

1. Appellants II and III argue that claim 1 lacks novelty over either document D1 or document D8.
2. *Novelty assessment vis-à-vis document D1*
  - 2.1 Document D1 is an international patent application published on 5 October 1995. A European patent application with application number 95 913 062.6 designating in particular the same Contracting States as the patent, namely BE, DE, DK, FR, GB and NL, was derived therefrom. It has the filing date of 29 March 1995 and claims three priorities, the earliest with a date of 29 March 1994. As the application, on which the patent was granted, was filed on 19 May 1994 and claims a priority of 19 May 1993, the content of document D1 is to be taken into consideration for novelty under Article 54(3) EPC only if the patent is not entitled to its priority date and provided that document D1 is entitled to its priority date of 29 March 1994. It is therefore appropriate to determine firstly whether the patent is entitled to the priority date and secondly, in the case of a negative answer, whether any of the

technical features of an  $\alpha$ -amylase according to claim 1 is missing in the disclosure of document D1.

2.2 An essential technical feature of the  $\alpha$ -amylase according to claim 1 is the presence of a sequence of Asn-Gly-Thr-Met-(Met)-Gln-Tyr-Phe-Glu-Trp in its N-terminal amino acid region. This specific feature is ignored in the priority document. The argument that it is a sequence present in all liquefying  $\alpha$ -amylases and thus inherent also in the enzyme of the priority document is not tenable in view of its specificity (primary sequence, length, optional methionine, etc..). Thus, the European application and the priority document are not for the same invention, the patent is not entitled to the priority date and its relevant filing date is 19 May 1994. Thus, subject to an assessment whether it is entitled to the priority date of 29 March 1994, document D1 is comprised in the state of the art for consideration of novelty (under Article 54(3) EPC).

2.3 Document D1 describes  $\alpha$ -amylases which are alkaline (see page 11, line 6), i.e. capable at alkaline pH values of hydrolysing 1,4- $\alpha$ -glucosidic linkages in starches, amylose, amylopectin and partial degradation products thereof and forming glucose (G1), maltose (G2), maltotriose (G3), maltotetrose (G4), maltopentose (G5) and maltohexose (G6) from amylose (see document D16, page 41 as a whole). As commented in detail on page 34, one of those  $\alpha$ -amylases, which was obtained from the *Bacillus* strain NCIB 12289 (see lines 4 to 5), has an isoelectric point of about 8,8 to 9,0 (see lines 6 to 7), has an optimum pH at pH 7,5 to 8,5 (see line 17) and includes in its N-terminal region the sequence

Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp (see line 23 to 25).

2.4 However, document D1 fails to give any indication as to the pullulanase activity, if any, of this particular  $\alpha$ -amylase which shares with an  $\alpha$ -amylase according to claim 1 all other technical features. There is no evidence on file beyond any doubt that the  $\alpha$ -amylase obtained from Bacillus strain NCIB 12289 does not act on pullulan.

2.5 Appellants II and III argue, without the support of any evidence possibly based on experimental results, that it is an inherent feature for an  $\alpha$ -amylase not to have a pullulanase activity and that, therefore, the enzyme obtained from the Bacillus strain NCIB 12289 is not acting on pullulan. The argument is not tenable in view of the fact that the prior art document D10 describes an enzyme having both amylase and pullulanase activities (see page 3, lines 11 to 36).

2.6 Thus, it has not been established that the liquefying  $\alpha$ -amylase obtained from the Bacillus strain NCIB 12289 is not acting on pullulan. Under these circumstances, it is considered that document D1 does not describe an  $\alpha$ -amylase according to claim 1. There is thus no need to discuss priority entitlement of document D1, because the subject-matter of claim 1 is in itself new over document D1.

### 3. *Novelty assessment vis-à-vis document D8*

3.1 Document D8 belongs to the state of the art as defined in Article 54(2) EPC. It describes the nucleotide

sequence of the gene for an amylase obtained from an alkalophilic Bacillus strain (referred to as Bacillus sp. #707) and the amino acid sequence deduced therefrom (see Figure 2 on page 28 and the accompanying legend on page 29). That sequence includes in its N-terminal region the sequence Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp. The authors suggest that this amylase is a liquefying  $\alpha$ -amylase (see page 31, last paragraph).

- 3.2 Document D8 fails to give any further characterisation of the enzyme, in particular as regards its isoelectric point, its optimum pH and its pullulanase activity, if any.
- 3.3 Appellant II has provided a scientific report (see document D34) in which amylases as obtainable from Bacillus sp. #707 (the amylase of document D8) and from Bacillus KSM-AP1378 (the amylase of the patent) have been characterised. The conclusion is reached at the end of the report that the amylase of document D8 has only very minute activities on pullulan, an isoelectric point between 8.7 and 9.0, and a pH optimum around 9.0.
- 3.4 At first glance, those results would support appellant II's and appellant III's position on lack of novelty, provided that, as argued in the declaration of Carsten Andersen (document D37; see points 4 to 8), the finding that the enzyme of document D8 has only very minute activities on pullulan means that it does not act on pullulan.
- 3.5 Appellant I argues, however, that the results provided in document D34 are not credible as the experiments

were not performed in conformity with the usual quality standards of the technical field in question. In particular, appellant I points out that the gel represented in the last page of document D34 shows not one, as normally expected, but two isoelectric point bands for each of the amylases tested, which may reflect the use of insufficiently purified enzyme preparations. The admission by appellant II at the oral proceedings that the results given in document D34 are an average reflecting the results obtained after having performed in triplicate the reported experiments, whereas this is not mentioned in document D34, renders the situation more confusing.

3.6 Taking into account these remarks, the Board comes to the conclusion that the data contained in document D34 in relation to certain parameters do not meet the high level standards of quality expected from data on the basis of which it has to be decided whether a claimed invention is new over a document such as document D8 in which those parameters are not referred to. In other words, the data of document D34 do not permit a person skilled in the art to perform a reliable novelty assessment.

4. Thus, it has not been established that the amylase obtained from Bacillus sp. #707 as referred to in document D8 has each and every one of the technical features of an amylase according to claim 1. Therefore, in the Board's judgement, document D8 does not describe such an  $\alpha$ -amylase and, consequently, the subject-matter of claim 1 is new under Article 54 EPC.

*Inventive step (Article 56 EPC)*

5. As to the determination of the closest state of art, it has to be decided which of document D5, as chosen by appellant I and the opposition division, or document D8, as proposed by appellants II and III, is the relevant one.
  
6. As explained at point 3.1 (see supra), document D8 provides a preliminary characterisation of an amylase which appears to share some of the technical features of an amylase according to claim 1: i) it is produced by a Bacillus strain, ii) its amino acid sequence as deduced from the coding nucleotide sequence shows that it contains at its terminal amino region the sequence Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp and iii) the authors suggest it is a liquefying  $\alpha$ -amylase.
  
7. Document D5, which is an abstract of a Japanese patent application, briefly describes an amylase which is also produced from a microorganism belonging to the genus Bacillus. It is indicated that it has an optimum pH at 10, a value which is comprised within the pH range referred to in claim 1. None of the other technical features contained in claim 1 are mentioned. On the other hand, it is acknowledged to have an isoelectric point of 4,3, i.e. an acidic value, in stark contrast with the alkaline range of pI values indicated in claim 1. This suggests that the amylase of document D5 is not directly related to an amylase according to claim 1.



8. As the closest prior art for assessing inventive step is normally a prior art document disclosing subject-matter having the most relevant technical features in common, not D5 but D8 is the relevant document.
  
9. In view of document D8, the technical problem solved by the invention is regarded as the provision of a further liquefying  $\alpha$ -amylase, the solution to that problem being an amylase according to claim 1.
  
10. The question to be answered for the assessment of inventive step is whether a person skilled in the art facing that technical problem would have been prompted by any prior art document to look for an amylase which, in addition to the technical features mentioned in document D8 (see point 6, supra), would not act on pullulan and would have both an isoelectric point of 8,7 to 9.7 and an optimum pH pf from 8,0 to 10.
  
11. None of the prior art documents relied on by appellants II and III at the oral proceedings other than document D5, which would have taken the skilled person away from the invention in view of the acidic isoelectric point referred to therein, namely documents D6, D9, D10, D11 and D28, would have been helpful to the skilled person for the following reasons:
  - 11.1 Each of documents D6, D10 and D11 relates to an alkaline pullulanase (see document D6, column 2, lines 59 to 67; document D10, page 3, lines 23 to 31; and document D11, page 3, lines 19 to 28 and 47 to 53), which is in stark contrast with an amylase according to claim 1 which does not act on pullulan.

- 11.2 Document D9 relates to mutated subtilisin proteases, whereas claim 1 is directed to an amylase.
- 11.3 Document D28 is a review discussing alkaliphilic bacteria. The mere statements on page 354, especially referred to by appellant II, according to which "[P]roteases and amylases are the most widely used enzymes" and "[D]etergents usually have a pH in solution between 8 and 10,5" are insufficient to provide the skilled person with the necessary guidance to arrive at an amylase as featured in claim 1, in particular as regards the value of the isoelectric point.
12. Therefore, it is concluded that claim 1 involves an inventive step. Thus, the main request meets the requirements of Article 56 EPC.

*Sufficiency of disclosure (Article 83 EPC)*

13. Appellants II and III argue that the claimed invention is not disclosed in a manner sufficiently clear and complete for it to be reproduced by a person skilled in the art.
14. The Board notes that a sample of the biological material referred to as Bacillus sp. KSM-AP1378, from which the exemplified enzyme has been obtained, has been deposited with a recognised depositary institution in accordance with the provision of Rule 31 EPC (former Rule 28 EPC 1973). This has not been contested by either of appellants II and III. As a result, that biological material has been made available, under the

- accession number FERM BP-3048, to the skilled person who, therefore, is in a position to obtain and test the exemplified enzyme.
15. The deposit of Bacillus sp. KSM-AP1378 renders untenable the argument that the disclosure is insufficient due to the absence of any indication in the patent how that particular strain was found.
  16. The question to be answered for the present assessment is whether in view of the disclosure of the invention in the patent the skilled person is in a position to establish that amylases he/she might retrieve from other Bacillus strains have the technical features referred to in claim 1 and/or claim 2.
  17. All the parameters to be measured as regards those technical features are common knowledge. Measuring an isoelectric point or measuring an enzymatic activity does not require unusual skills for the skilled person.
  18. The objections made by appellants II and III regarding features b) and c) of claim 1 and features c), f), i) and j) of claim 2 are not convincing:
    - 18.1 As regards feature b) of claim 1, the skilled person is informed in paragraph 0022 of the patent (see page 5) that "*not acting on pullulan*" means that at a pullulan concentration of 0,25% the relative activity of the amylase on that substrate should be 0,0, while when measured in parallel the relative activity on other substrates tested at the same concentration of 0,25% should have the values indicated in Table 1. This is a clear and complete disclosure.

- 18.2 As regards feature c) of claim 1, appellants II and III complain that the method used for measuring the isoelectric point has not been indicated. This is not the case as in various places in the patent (see paragraphs 0012, 0017, 0028 and 0082 on pages 4 to 6 and 19, respectively) reference is made to isoelectric focusing electrophoresis, a standard method for this purpose.
- 18.3 As regards features c), f) and i) of claim 2, the skilled person would appreciate by experience what the required stability is.
- 18.4 The objection to the undetermined terms "extremely" and "substantially" as regards features i) and j) of claim 2, to the use of parentheses for the second methionine in the amino acid sequence of claim 1 as well as to the expression "derived from" in claim 1 is more a lack of clarity objection (see Article 84 EPC), which is not applicable to claims 1 and 2 as granted, than an objection of insufficient disclosure.
19. In view of the above remarks, it is concluded that the claimed invention is disclosed in the patent in a manner sufficiently clear and complete. Thus the main request meets the requirements of Article 83 EPC.

**Order**

**For these reasons it is decided that:**

1. The decision under appeal is set aside.
2. The patent is maintained as granted.

The Registrar

The Chairman

A. Wolinski

L. Galligani