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Language of the proceedings: EN

Title of invention:
RECOVERY OF INSOLUBLE ENZYME FROM FERMENTATION BROTH AND FORMULATION OF INSOLUBLE ENZYME

Patent Proprietor:
Danisco US Inc.

Opponent:
Novozymes A/S

Headword:
Method of preparing an enzyme containing granular formulation/ DANISCO

Relevant legal provisions:
EPC Art. 54, 56

Keyword:
"Main Request - requirements of the EPC met (yes)"

Decisions cited:

Catchword:

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Case Number: T 2213/18 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 3 May 2022

Appellant: (Patent Proprietor)	Danisco US Inc. 925 Page Mill Road Palo Alto, CA 94304 (US)
Representative:	Forrest, G. Vaughan, N. Mewburn Ellis LLP Aurora Building Counterslip Bristol BS1 6BX (GB)
Appellant: (Opponent)	Novozymes A/S Krogshøjvej 36 2880 Bagsværd (DK)
Representative:	Pugh, R. Hoffmann, B. Potter Clarkson The Belgrave Centre Talbot Street Nottingham NG1 5GG (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 3 August 2018 concerning maintenance of the European Patent No. 2285823 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	A. Bacchin

Summary of Facts and Submissions

I. European patent No. 2 285 823 is based on European patent application No. 09763466.1, which was published as WO 2009/152176 under the Patent Cooperation Treaty (hereinafter "the patent application"). The patent was opposed on the grounds of Article 100(a) EPC in conjunction with Articles 54 and 56 EPC, and of Article 100(c) EPC. An opposition division considered the main request and auxiliary request 1 to lack novelty and decided to maintain the patent in amended form on the basis of auxiliary request 2.

II. The patent proprietor (appellant I) and the opponent (appellant II) lodged an appeal against the decision of the opposition division to maintain the patent on the basis of auxiliary request 2.

III. With its statement of grounds of appeal, appellant I submitted a main request and a first auxiliary request. The main request corresponds to patentee's main request submitted on the 18 July 2017 but without the product claims 6 to 10 and 14 while the first auxiliary request corresponds to the second auxiliary request considered allowable by the opposition division. In reply to appellant II's statement of grounds of appeal, appellant I submitted second to fifth auxiliary requests, identical to the third to sixth auxiliary requests submitted on 11 April 2018.

IV. The parties were summoned to oral proceedings. In a communication pursuant to Article 15(1) RPBA 2020, the parties were informed of the board's provisional, non-binding opinion, inter alia on issues concerning Articles 123(2)(3), 84, 54 and 56 EPC.

V. Oral proceedings were held by video conference on 3 May 2022 in the presence of both parties.

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

"1. A method of making an enzyme-containing granular formulation, comprising: recovering insoluble enzyme from a microbial broth comprising microbial cells that express the enzyme and/or cell debris from microbial cells that express the enzyme, without removing the microbial cells and/or cell debris, thereby producing a composition comprising recovered insoluble enzyme and microbial cells and/or cell debris, wherein at least some of the enzyme is insoluble in the microbial broth; producing an enzyme-containing granule that comprises said composition, wherein the enzyme is enzymatically active in the granule."

Dependent claims 2 to 8 define specific embodiments of claim 1.

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

D1: WO 01/25411 A1 (published 12 April 2001);

D3: WO2004/003187 A2 published 8 January 2004);

D8: US 6 316 240 B1 published 13 November 2001);

D11: A.L. Demain, P. Vaishnav "Production of recombinant proteins by microbes and higher organisms". Biotechnology Advances, vol. 27(3), pages 297-306 (Epub 31 January 2009).

VIII. The submissions made by the Proprietor/Appellant I, insofar as relevant to the present decision, are summarized as follows:

Main request

Claim interpretation with respect to the term "insoluble enzyme" in claim 1.

The "insoluble enzyme" is defined in paragraphs [0075], [0085] and [0091] of the patent (see decision under appeal, page 5, penultimate paragraph).

Novelty (Article 54 EPC)

Document D1 described that the starting material for processes of the invention was a fermentation broth or an enzyme containing liquid depending on the steps of the process (see page 11, lines 6 to 9).

In a first aspect, the starting material was a fermentation broth comprising microbial cells and/or cell debris thereof (page 11, line 10 to page 12, line 9). In a second aspect, the starting material was an enzyme containing liquid and the process comprised the step of spray drying the enzyme containing liquid to obtain a first dry enzyme-containing particle and subsequently subjecting the first dry particle to a second process step to obtain a second dry enzyme containing particle (see page 12, lines 10 to 15). The starting material was an aqueous liquid, such as an aqueous solution or dispersion of one or more enzymes, or a fermentation broth or a fermentation broth, which had been subjected to one or more processing steps (see page 12, lines 16 to 19 and lines 20 to 24). Reference was made to the definition of fermentation filtrate and

enzyme concentrate (see page 3, lines 10 to 18). The aqueous liquid and the dispersion defined embodiments where the biomass had been removed in the context of a starting material which contained biomass.

The starting material was presented as separate dependent embodiments and did not clearly and unambiguously disclose a fermentation broth containing a dispersion of one or more enzymes (see page 12). The method according to claims 12 and 13 of document D1 did not refer to an enzyme dispersion and for this reason could not anticipate the method of claim 1.

There was a very strong implication that the enzyme was soluble in the fermentation broth and certainly no explicit disclosure that the fermentation broth had to contain an insoluble enzyme. The use of Rodalon (50% Benzalkoniumchlorid) lysed the cells and released the enzyme to the culture medium. The insoluble enzyme was defined in [0075] of the patent and had to be interpreted with a mind willing to understand.

Inventive step (Article 56 EPC)

Document D1 represented the closest prior art.

It disclosed a process for preparing a particle comprising spray drying an enzyme and biomass containing fermentation broth starting material, to obtain a solid particle comprising an enzyme and a biomass, and a process for preparing an enzyme containing particle comprising spray drying an aqueous enzyme containing liquid starting material to obtain a spray dried first enzyme containing particle and subjecting the first dry particle to a process selected from granulation and coating and combinations thereof

to obtain a second dry enzyme containing particle (see abstract).
Since the biomass could be removed from a fermentation broth to provide a fermentation filtrate, using known methods such as filtration, centrifugation, flocculation and combinations thereof (page 12, 5th paragraph) whilst some of the biomass could be removed from a fermentation broth before spray-drying to optimize the broth properties and suitability for spray-drying, e.g. to adjust viscosity (page 11 lines 14-17), the enzyme had to be in soluble form, otherwise the enzyme would be removed together with the biomass. The low levels of expression in the region of g/L were such that the enzyme was expected to be soluble in the broth (page 11 lines 14 to 17; page 12 lines 16 to 28; page 15, lines 9 to 18). The problem of insoluble enzymes secreted into the growth medium was not addressed in document D1.

The difference between the teaching of document D1 and the method according to claim 1 was that the enzyme is secreted into the growth medium and is insoluble under the conditions used for growth of the cells.

The technical problem could be formulated as how to deal with insoluble enzyme in the microbial broth during the recovery process in the method for the production of enzyme-containing granular formulations.

There was nothing to direct the skilled person to apply the recovery methods from a fermentation broth where an enzyme secreted into that fermentation broth was insoluble.

Documents D3 and D8 proposed to keep the expressed enzymes in high yield in solution or to re-solubilise them respectively.

The protein concentration in document D1 on page 15 was representative. There was no indication why the concentration in mg/L should be erroneous and why the method could only be carried out when the protein concentration was in the g/L range.

A high product titer protease fermentation broth, with a dry matter content of 13% w/w, was reported in example 1 (see document D1, page 38). The high titer protease could not be assigned any specific concentration. Despite the indication that the fermentation broth had a dry content of 13% w/w, there was no indication of what proportion of this dry content was to be attributed to the enzyme expressed in the fermentation broth, let alone whether the enzyme was insoluble or not.

Document D11 related to the production of recombinant proteins which were inter alia produced in *E. coli* as inclusion bodies and necessitated a step of re-solubilizing and a refolding (see page 299, col.1, last paragraph and col.2, fourth paragraph).

Starting from document D1, there was no indication why the skilled person should turn to documents disclosing fermentation broths with high enzyme titers, as they already contained sufficient amounts of enzymes (see page 15, line 8). There was even less evidence that the enzyme was insoluble.

Although documents D1 and D3 expressed an amylase, the conditions, the hosts and the media were a priori

different. Starting from document D1, the skilled person found no motivation to modify the method of making an enzyme neither in document D1 nor in document D3 to arrive at the method according to claim 1.

IX. The submissions made by the Opponent/Appellant II, insofar as relevant to the present decision, may be summarized as follows:

Novelty (Article 54 EPC)

Document D1 disclosed a method of making enzyme granules, wherein the starting material to be dried was an enzyme in solid form (i.e. a dispersion) and the fermentation broth was directly spray dried without removal of cells and/or cell debris (see page 12, lines 10 to 24). The fermentation broth included cells and/or cell debris (see page 11, lines 12 to 14). The process for preparing a particle required spray drying of a fermentation broth or of an aqueous liquid which was then subjected to a process of granulation or coating (see page 2, lines 5 to 15). The fermentation broth covered an aqueous composition (see page 2, lines 25 to 28). The biomass removal from the fermentation broth was, if at all, minimal. The processes paired a high enzyme concentration in the fermentation broth to the possibility of directly spray drying the fermentation broth without removal of biomass (see page 15, lines 6 to 12). A "high product titre" (i.e. high enzyme concentration) fermentation broth was directly spray dried without removal of biomass (see examples 1 to 3).

Paragraph [0075] of the patent defined an insoluble enzyme as an enzyme that "... separates (i.e., partitions) with a solid phase upon separation of solid

and liquid phases, for example, solid and liquid phases of a microbial broth."

The starting material used in document D1 contained a certain minimum concentration of enzyme in the liquid and/or the enzyme constituted a certain minimum percentage of the solids (i.e. non-volatile components) in the liquid in order to produce particles by the process of the invention (see page 15, lines 1 to 18).

The second and third paragraphs of the second aspect on page 12 of document D1 were linked. They referred to the physical form of the starting material and to the nature of the starting material used: a fermentation broth, which according to the definition on page 2 was an aqueous composition.

The process in document D1 was defined in claim 13 referring back to claim 12. The aqueous enzyme containing liquid consisted of a fermentation broth, enzyme filtrate or enzyme concentrate. Even if some large solid particulates were removed and the fermented micro-organism was killed, the resulting broth was spray dried. This yielded small particles comprising active enzymes (see example 1).

Inventive step (Article 56 EPC)

Document D1 represented the closest prior art.

It disclosed the use of a high product titre fermentation broth, of a dispersion of the enzyme and of a direct spray drying of a fermentation broth containing biomass which was entirely compatible with the presence of dispersed enzyme. The purpose described

in document D1 and paragraph [0016] of the patent were the same.

The difference between the process of claim 1 and of document D1 was that the enzyme in the examples was not explicitly disclosed in a dispersed form.

The patent provided no data supporting an improvement over the method described in document D1.

The objective technical problem was therefore the provision of an alternative process to those described in examples 1 to 3.

The solution proposed by claim 1 was obvious on the basis of the content of document D1 alone.

The skilled person reading document D1 would have immediately noted that the reported enzyme concentrations were low. They were erroneous and had to be read as g/L instead of mg/L (see page 15, lines 1 to 21). This view was confirmed by the disclosure in document D11 and paragraph [0010] of the present patent). These expression levels were known in the art.

The broth was specified to contain 10 to 15% w/w dry matter while the enzyme constituted up to 50% w/w of the dry matter, preferably up to 10% w/w of the dry matter (see document D1, page 11, line 18; page 38, example 1). Thus, if the fermentation broth contained 10% w/w dry matter and the enzyme constituted 10% w/w of the dry matter, then the enzyme constituted 1% w/w of dry matter of the fermentation broth corresponding to a concentration of 10 g/L. This confirmed as well that the reported concentration was erroneous and had to be read as g/L.

There was nothing surprising to get undissolved enzyme in a fermentation broth when the enzyme production methods became so efficient and high yielding that an enzyme was expressed at levels above its solubility limit. Document D3 reported that enzymes produced at high yields in fermentation broth might contain insoluble polypeptide precipitate in the form of crystals or amorphous precipitate (see background art section). The solution was to prevent crystallisation and precipitation of the enzyme by adding a carbohydrate or polyol to the culture solution (see page 2, lines 22 to 27). Since both documents D1 and D3 produced a high titer of amylase, the skilled person knew that, based on the common general knowledge (see background art of document D3), should the polypeptide be expressed at high expression yields e.g. above its solubility limit, it would crystallise and precipitate. Under such circumstances, the skilled person would also follow the instructions provided in document D1 and directly spray dry the fermentation broth containing an insoluble polypeptide, thereby arriving at the method of claim 1.

X. Appellant I requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request, or alternatively on the basis of one of the above indicated first to fifth auxiliary requests.

XI. Appellant II requested that the decision under appeal be set aside and that the patent be revoked.

Reasons for the Decision

Main request (claims 1-8)

1. The main request is identical to the first auxiliary request underlying the decision under appeal, but without the product claims 6 to 10 and 14.

2. Claim 1 reads:

A method of making an enzyme-containing granular formulation comprising:

recovering insoluble enzyme from a microbial broth comprising microbial cells that express the enzyme and/or cell debris from microbial cells that express the enzyme, without removing the microbial cells and/or cell debris, thereby producing a composition comprising recovered insoluble enzyme and microbial cells and/or cell debris, wherein at least some of the enzyme is insoluble in the microbial broth;

producing an enzyme-containing granule that comprises said composition, wherein the enzyme is enzymatically active in the granule.

3. The "insoluble enzyme" is defined in paragraphs [0075], [0085] and [0091] of the patent.

- [0075]: "An insoluble enzyme refers to an enzyme that is present in a solid phase. An insoluble enzyme separates (i.e. partitions) with a solid phase upon separation of solid and liquid phases, for example, solid and liquid phases of a microbial broth. It is understood that in a whole microbial broth some enzyme will typically also be found in the soluble phase in the medium in addition to the insoluble enzyme that is part of

the solid phase. The insoluble enzyme may be bound to solids in the microbial broth, such as cell solids or other solid components or may be precipitated or crystallized within the microbial broth."

- [0085]: "In one embodiment, ... at least some of the enzyme is insoluble ... . In another embodiment, the expressed enzyme is soluble in the microbial broth, and at least some of the enzyme is rendered insoluble by addition of one or more precipitant(s)."

- [0091]: "In various embodiments, any of at least 90, 80, 70, ... 10% of the enzyme is insoluble ... ."

3.1 It needs to be established what is encompassed by the step of recovering an "insoluble enzyme".

3.1.1 In the board's view there is no limitation in claim 1 to fermentation broths comprising only insoluble enzyme. As long as at least some of the enzyme in a broth is insoluble, i.e. separating with cells or cell debris upon separation of solid and liquid phases, it is encompassed by this term. A dispersion of enzymes is considered to fall under the definition of a microbial broth comprising an insoluble enzyme wherein at least some of the enzyme separates (i.e.,partitions) with a solid phase upon separation of solid and liquid phases.

3.1.2 An enzyme produced intracellularly and not yet secreted will at least partially separate with a solid phase upon separation of solid and liquid phases - for example, solid and liquid phases of a cell culture broth - It follows that the recovered fermentation

broth comprising both an enzyme and the microbial cells and/or cell debris will necessarily and inevitably comprise at least some insoluble enzymes.

4. Appellant I stressed that the method of claim 1 had to comprise the step of recovering the insoluble enzyme from a microbial broth comprising microbial cells, ... without removing the microbial cells and/or cell debris, thereby producing a composition comprising recovered insoluble enzyme and microbial cells and/or cell debris, wherein at least some of the enzyme is insoluble in the microbial broth.

4.1.1 It submitted that the sole meaningful interpretation for the step of recovering the insoluble enzyme from a microbial broth in claim 1 was that the enzyme from the microbial broth was brought into contact with at least a solvent to allow the enzymes to partition with the soluble and/or insoluble phase. Even if the solubility of the said enzyme in the solvent varies from completely insoluble to completely soluble, it was clear that small amounts of enzymes defined as completely soluble would nevertheless separate with the solid phase. Hence, any interpretation which attributed to an enzyme from a microbial broth the status of insoluble enzyme separating with the solid phase because it was encapsulated in the cells rendered this feature redundant and meaningless, as enzymes encapsulated in cells were incapable of separating with the liquid phase.

5. The board agrees with appellant I's view that the method of claim 1 also comprises a step of recovering insoluble enzyme from a microbial broth comprising microbial cells. An insoluble enzyme encapsulated in the cells cannot be recovered from a microbial broth.

This interpretation is in line with the definition of microbial broth in paragraph [0074] of the patent: *"Microbial broth" or "fermentation, broth" refers to a growth medium in which microbial (e.g., bacterial or fungal) cells are grown and which is suitable for expression of an enzyme as described herein*.

Novelty (Article 54 EPC)

6. The decision under appeal considered that document D1 described two different starting materials for the processes of the invention. In a first aspect, the starting material consisted of a fermentation broth whereas in the second aspect it consisted of an enzyme containing liquid, such as an aqueous solution or a dispersion of one or more enzymes. Even if document D1 provided no definition for the term dispersion of enzymes, it covered insoluble enzymes precipitated in an aqueous solution when they were bound to solids in the microbial broth, such as cell solids. Claim 13 defined further that the aqueous enzyme liquid was a fermentation broth, an enzyme filtrate or an enzyme concentrate, wherein the aqueous liquid embraced preferably an aqueous solution or dispersion of one or more enzymes.

6.1 Appellant II contended that document D1 disclosed that the invention related to overlapping first and second aspects. The enzyme in solid form (i.e. a dispersion) and the fermentation broth were directly sprayed without removal of cells and/or cell debris. The fermentation broth included cells and/or cell debris.

6.2 The board notes that there is no dividing line between fermentation broths and aqueous compositions or aqueous enzyme containing liquids (see document D1, page 2,

lines 25 to 28; page 12 lines 10 to 24; claims 12 and 13). Document D1 mentions that "[W]e have surprisingly found it possible to provide fermentations which directly yields fermentation broths having a sufficiently high enzyme content, so that the broth may be dried directly or only minimally refined by removing biomass, sterilization and addition of additives to obtain a dry powder having an enzyme content sufficiently high to give an enzyme product useful in most applications." (see page 15, lines 6 to 12). After an enzyme containing fermentation broth was sieved through a rotary brush strainer and the fermented microorganism was killed, a high product titre protease, cellulase or amylase fermentation broth was directly spray-dried without removing the biomass (see examples 1 to 3).

The board considers that in the first aspect of the invention, the starting material is a fermentation broth, and the process comprises the step of spray drying said fermentation broth, even if some of the biomass may be removed before spray drying to optimize the broth properties and suitability for spray drying. Document D1 does not explicitly refer to a dispersion of one or more enzymes, wherein the enzyme is insoluble in the microbial broth - a growth medium in which microbial cells are grown. Furthermore, there is no evidence that the fermentation broth used as starting material in the process specified in the first aspect of the invention must specifically comprise a dispersion of enzymes which is only described as one of the preferred aqueous liquids falling under the second aspect of the invention.

6.3 Appellant II submitted that in the second aspect of the invention the starting material was an enzyme

containing liquid and the process comprised the step of spray drying said enzyme containing liquid. The starting material to be dried could be a dispersion of enzyme (see document D1, page 12). In a preferred embodiment of this second aspect of the invention, the starting material was a fermentation broth or a fermentation broth which had been subjected to one or more processing steps, such as fermentation filtrate or enzyme concentrate. The first two sentences related to the physical form of the starting material, which included a dispersion of enzymes, while the third sentence related to the nature of the starting material. They were linked.

6.4 In the board's view there is no direct and unambiguous disclosure that the enzyme containing liquid used in the process set out in the second aspect of the invention is a fermentation broth comprising a dispersion of one or more enzymes which has not been subjected to processing steps. Moreover, the dispersion is only one of the aqueous liquids defined as preferred enzyme-containing liquids.
Even if the second aspect of the invention is mentioned to be preferably an aqueous liquid such as an aqueous solution or dispersion of one or more enzymes, it is only in a preferred embodiment that the starting material is defined as a fermentation broth or a fermentation broth that has been subjected to one or more processing steps. Hence, there is no direct and unambiguous disclosure in this section of document D1 of a process comprising a step of recovering an insoluble enzyme from the microbial broth comprising microbial cells without removing the microbial cells and/or cells debris.

6.4.1 Even if claims 12 and 13 confirm that the aqueous enzyme containing liquid in the second aspect of the invention may be a fermentation broth, the methods set out in claims 12 and 13 do not refer to an enzyme dispersion, let alone to a combination of a fermentation broth that is containing an insoluble enzyme. Since the second aspect of the invention discloses neither directly nor unambiguously a step of spray drying a fermentation broth comprising cells and/or cell debris, which has not been subjected to one or more processing steps, and contains a dispersed enzyme, claims 12, 13 and the second aspect of document D1 do not anticipate the method of present claim 1.

6.5 Referring to page 15, appellant II stressed also that the starting material had to contain a minimum concentration of enzyme in the liquid and/or the enzyme constituted a certain minimum percentage of the solids (i.e. non-volatile components) in the liquid in order to produce particles by the process of the invention, which have a useful enzyme content.

6.5.1 In the board's view this section of document D1 neither directly nor unambiguously discloses that the enzyme in the liquid is insoluble and/or that the minimum percentage of the solids (i.e. non-volatile components) in the liquid contains an insoluble enzyme.
The minimum percentage of the solids in the liquid, i.e. the non-volatile components, can only mean that after the drying or spray drying of a sample (also called dry matter) there is a minimum amount of enzyme contained in the mass of solids. This section also fails to further specify that the method comprises the step of recovering an insoluble enzyme from a microbial broth comprising microbial cells without removing the microbial cells and or cell debris.

6.6 Hence, the method of claim 1 of the main request is not anticipated by the methods disclosed in document D1. The main request fulfils the requirements of Article 54 EPC.

Inventive step (Article 56 EPC)

7. Document D1 represented the closest prior art for appellant II, while documents D3 or D8 represented the closest prior art for appellant I.

7.1 The closest prior art is generally a document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most technical features in common with said invention requiring a minimum of structural modifications (see Case Law of the Boards of Appeal, 9th edition, Chapter I.D.3.2).

7.2 Appellant II submitted that document D1 discloses a process for preparing an enzyme-containing particle (i.e., a granule) by spray drying a fermentation broth (see page 2, lines 5 to 8). The fermentation broth and the particle comprise biomass (see definitions on page 2, lines 25 to 29 and page 3, line 6 to 8). It discloses insoluble enzyme in the starting material to be spray dried in which the enzyme constitutes a minimum percentage of the solids in the liquid. The enzyme in the granules is active (see page 14, line 29 to page 15, line 6; page 31, lines 10 to 24). The purpose described in document D1 and in paragraph [0016] of the patent is the same.

7.2.1 Document D3 relates to a method of increasing solubility of a polypeptide of interest during

fermentation. When the fermentation yields are getting higher and higher - due to optimization of the fermentation recipes and/or due to identification/ development or construction of more efficient production organisms, - the polypeptides are fermented above their solubility limit. The polypeptide may therefore be in the culture broth partly precipitated. This causes problems in recovery. Special measures have to be taken to solubilize the crystals/amorphous precipitate before removing the cells and other solids from the culture broth. These measures result in yield losses (see page 1, lines 8 to 17).

7.2.2 Document D8 discloses a method for recovering glycosidase or a peptidase from a culture broth, where a significant amount is not in a soluble state (see column 1, lines 13 to 16). A significant amount of insoluble enzyme can be recovered by the use of extreme pH values (see column 2, lines 19 to 34). Problems and solutions for the recovery of insoluble enzyme known in the art are mentioned (see column 1, lines 19 to 21 and 35 to 39).

7.3 Documents D3 and D8 address the problem of recovery of insoluble enzyme by re-solubilizing (D8) or by avoiding precipitation in the first place (D3).

7.4 The board, in agreement with appellant II, notes that neither document D3 nor D8 discloses enzyme granules or their preparation. Since they are not directed to the same purpose or effect as the claimed invention, i.e. making an enzyme granular formulation, documents D3 and D8 cannot qualify as the closest prior art.

Starting from document D1

8. In agreement with the decision under appeal, the board notes that the only difference between the teaching of document D1 and the method according to claim 1 is that there is insoluble enzyme in the growth medium.

8.1 In the board's view, based on the definition given in paragraph [0075] of the patent and the fact that at least about 10% to 90% of the enzyme is insoluble in the microbial broth prior to recovery (see paragraph [0091]), the microbial broth must comprise at least some insoluble enzyme in its extracellular compartment (growth medium) because said enzyme is either poorly soluble or partially insoluble because it is present at a concentration above its solubility limit.

8.2 Appellant II asserted that the enzyme became insoluble in the growth medium when the concentration exceeded a certain value. This interpretation was consistent with the definition given in paragraph [0075] and with the fact that at least about 10% of the enzyme is insoluble in the microbial broth prior to recovery (see paragraph [0091] of the patent). Claim 1 of the present invention was a process according to document D1 in which the enzyme was produced in high amounts above its solubility limit.

8.2.1 The only difference between the method described in document D1 and the method according to claim 1 was that the enzyme was not explicitly disclosed to be insoluble in the growth medium.

8.3 With regard to the content of document D1, appellant I asserted that the technical problem was how to recover insoluble enzyme from the microbial broth. This situation was encountered when the enzyme was produced

in a fermentation broth at high yield above its solubility limit.

8.4 In agreement with appellant II and in the absence of any evidence for an improvement over the method of document D1, the board defines the objective technical problem as the provision of an alternative process for making an enzyme containing granular formulation.

8.5 The method according to claim 1 solves this problem.

8.6 Appellant II argued that the "very low" enzyme concentrations in mg/L reported in document D1 at page 15, lines 15 to 18 were immediately recognizable by the skilled person as erroneous.

Document D1 referred to a fermentation broth comprising a biomass, to the percentage of biomass and to the dry-matter in percent w/w present in this biomass. Even if the skilled person considered a fermentation broth with the lowest biomass proportion (10%) and the lowest percentage of dry-matter in the biomass (10%), the fermentation broth had a calculated insoluble enzyme content of 1%, which corresponded to an insoluble enzyme concentration of 10 g/L in the microbial broth. Thus, the reported concentration in mg/L was a typographical error which would immediately have been replaced by the skilled person by the correct concentration level in g/L. The correction was supported by the background art of the present patent and the review document D11 and was consistent with the references in document D1 to high enzyme content and high product titre (see [0010] of the patent and document D1, page 15, line 8 and the examples respectively).

8.6.1 Applying high expression techniques in fermentation lead inevitably to the production of insoluble enzymes.

Since document D1 contemplated the spray-drying of fermentation broth and enzyme containing liquid such as dispersion of one or more enzymes, the skilled person would simply have followed the instructions provided in document D1 and directly have spray dried the fermentation broth containing an insoluble polypeptide, thereby arriving at the method of claim 1.

9. The board considers first that there is no compelling evidence for appellant II's assertion that the values in [mg/l] for the enzyme expression levels in document D1 were incorrect and had to be [g/l]. Neither an error nor its only possible correction are immediately evident. Thus, this assertion cannot stand. Starting from the teaching of document D1, the skilled person had therefore no motivation to select fermentation broths yielding expression levels orders of magnitude higher than those disclosed in document D1 (see page 15, first paragraph).

9.1 Moreover, even if document D1 discloses that in a preferred embodiment the fermentation broth comprises at least 10% of the biomass and the broth comprises at least 10 to 15% w/w dry matter and the biomass constitutes up to 10% w/w of the dry matter, the resulting 1% w/w of biomass dry matter in the fermentation broth is nowhere disclosed as consisting exclusively of enzyme, let alone insoluble enzyme.

9.1.1 The skilled person would therefore take the reported product titers at face value.

9.2 Although the case law defines the skilled person as being cautious and having a conservative attitude (cf. "Case Law", supra, I.D.8.1.3, page 207), it also acknowledges that obvious developments of the state of the art belong to the normal tasks of the skilled person and that routine adaptations as well as the use of known alternatives do not go beyond what may normally be expected from a skilled person.

9.3 The board considers that document D1 discloses nowhere that enzymes - at the concentrations disclosed - may have poor or low solubility or that the polypeptide could be expressed at high expression levels, i.e. above its solubility limit, which would result in its crystallisation and precipitation. Hence, document D1 provides no motivation to recover insoluble enzymes from a microbial broth comprising microbial cells without removing the microbial cells and/or cell debris.

9.4 Although the skilled person could use fermentation protocols capable of obtaining higher concentrations of enzymes, i.e. above their solubility limits, document D1 provides no incentive to do so.

9.4.1 Even if, arguendo, the skilled person were to choose to express enzymes at high yield, above their solubility limits, the skilled person would turn to documents D3 and D8 which already addressed this problem and kept the expressed enzymes in solution or re-solubilised them.

9.4.2 Even if document D1 contemplates the spray-drying of fermentation broth and enzyme containing liquid such as dispersion of one or more enzymes, it does not disclose or suggest that the method of preparing an enzyme

particle could alternatively comprise the step of first expressing the enzyme at a higher yield than disclosed, i.e beyond their solubility limits, in the microbial broth, so as to produce and recover soluble and insoluble enzymes or poorly soluble enzymes in the microbial broth, and second without removing the microbial cells and/or cell debris.

9.5 Although the starting material contains a certain minimum concentration of enzyme in the liquid and/or the enzyme constitutes a certain minimum percentage of the solids (i.e. non-volatile components) in the liquid, the percentage of solids in the liquid can only define a percentage of dry matter, irrespective of whether these solids are soluble or insoluble in the liquid (see document D1, page 15, lines 1 to 12). It follows that if the enzyme is recovered from relatively impure fermentation broth, which already contains cells and/or cell debris in solid form, by applying the method of the first aspect of the invention, the board cannot establish whether the enzyme in the microbial broth comprising the microbial cells was insoluble or not.

9.6 The board considers that even if the aqueous liquid in the second aspect of the invention described in document D1 can be a fermentation broth in the light of claims 12 and 13, and examples 1 to 3, which refer to spray dried powder obtained from fermented micro-organisms whose membrane had been disrupted, without removing the microbial cells and/or cell debris, there is no direct and unambiguous indication that any of the enzymes was insoluble in the microbial broth. Likewise, document D1 does not teach that the starting material in the process of the second aspect, which is an enzyme-containing liquid, preferably an aqueous liquid,

such as an aqueous solution or dispersion of one or more enzymes, is in fact a fermentation broth from which the microbial cells and/or cell debris have not been removed, which is/comprises also a dispersion of one or more enzymes. The mere general recommendation of minimising the removal of cells or cell debris set out in document D1, without excluding this removal, does not render the claimed method obvious.

9.7 The board disagrees with appellant II's view that the skilled person, faced with the technical problem of providing an alternative process would simply have followed the instructions provided in document D1 and directly have spray dried the fermentation broth containing an insoluble polypeptide, thereby arriving at the method of claim 1 with no difficulty.

9.7.1 Indeed, neither the first nor the second aspect of the invention establish that the enzyme is insoluble in the microbial broth and that it can be recovered from the microbial broth without removing the microbial cells and/or cell debris. Likewise, the percentage of total dry matter in a fermentation broth does not indicate what proportion of this dry content may be attributed to the enzyme expressed in the fermentation broth.

9.8 Starting with the first or second aspect of the invention described in document D1, the skilled person faced with the technical problem of providing an alternative method, had no motivation without a pointer to devise an alternative method of preparing an enzyme containing particle with the features of claim 1.

9.9 The method of claim 1 involves an inventive step.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent on the basis of claims 1 to 8 of the main request submitted on 12 December 2018 and a description to be adapted thereto.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated