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D E C I S I O N
of 2 December 1994

Case Number: T 0137/92 - 3.3.4

Application Number: 84306053.4

Publication Number: 0140542

IPC: C12N 11/08

Language of the proceedings: EN

Title of invention:
An immobilized lipase preparation and use thereof

Patentee:
NOVO NORDISK A/S

Opponent:
KAO Corporation

Headword:
Immobilized Lipase/NOVO

Relevant legal provisions:
EPC Art. 56

Keyword:
Inventive step - (yes)

Decisions cited:
-

Catchword:
-



Case Number: T 0137/92 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 2 December 1994

Appellant:
(Opponent)

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Respondent:
(Proprietor of the patent)

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Decision under appeal:

Decision of the Opposition Division of the
European Patent Office dated 3 December 1991
rejecting the opposition filed against European
patent No. 0 140 542 pursuant to Article 102(2)
EPC.

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: R. E. Gramaglia
S. C. Perryman

Summary of Facts and Submissions

- I. European patent No. 0 140 542 based on application No. 84 306 053.4 was granted on the basis of 17 claims, of which claim 1 and independent claim 14 read as follows:

"1. An immobilized lipase preparation for interesterification of fats, hydrolysis of fats and/or synthesis of fats without the use of non-aqueous solvent or other auxiliary agents used to control viscosity and solubility, which lipase is obtainable by contacting an aqueous solution of a microbial lipase with a particulate, macroporous, weak anion exchange resin which contains primary and/or secondary and/or tertiary amino groups and which exhibits a relatively large average particle size suitable for column operation without excessive pressure drop, at conditions, at which the lipase is bonded to the anion exchange resin during a period of time sufficient to bind the wanted amount of lipase to the anion exchange resin, thereafter separating the thus-formed immobilized lipase from the aqueous phase and drying the separated immobilized lipase to a water content of from 2 to 40%."

"14. A method of producing an immobilized lipase preparation for interesterification of fats, hydrolysis of fats and/or synthesis of fats, without the use of non-aqueous solvent or other auxiliary agents used to control viscosity and solubility, which method comprises contacting an aqueous solution of a microbial lipase with a particulate, macroporous, weak anion exchange resin which contains primary and/or secondary and/or tertiary amino groups and which exhibits a

relatively large average particle size suitable for column operation without excessive pressure drop, at conditions, at which the lipase is bonded to the anion exchange resin during a period of time sufficient to bind the wanted amount of lipase to the anion exchange resin, thereafter separating the thus-formed immobilized lipase from the aqueous phase and drying the separated immobilized lipase to a water content of from 2 to 40%."

II. Opposition was filed on the grounds of Article 100(a) and (b) EPC, on the basis of lack of novelty (Article 54 EPC), lack of an inventive step (Article 56 EPC) and insufficiency of disclosure (Article 83 EPC). Included among the many prior art documents introduced were the following:

- (1) US-A-4 170 696
- (5) US-A-4 275 081
- (8) Kimura et al., Eur. J. Appl. Microbiol. Biotechnol. 17, 107-112 (1983)
- (9) In Re USSN 080217
- (10) GB-A-1 577 933
- (11) EP-A-64 855
- (12) In Re USSN 646752
- (13) US-A-4 826 767
- (14) Mukherjee et al., Appl. Microbiol. Biotechnol. 35, 579-584 (1991)
- (15) Bilyk et al., JAOCS, 68, 320-323 (1991)
- (16) Iwai et al., J. Gen. Appl. Microbiol. 10, 13-21 (1964)

III. By its decision given orally on 24 October 1991, and issued in writing on 3 December 1991, the Opposition Division rejected the opposition and maintained the patent as granted.

- IV. An appeal against that decision was filed on 3 February 1992 with the payment of fee. A Statement of Grounds was filed on 1 April 1992.
- V. In a communication accompanying the summons to oral proceedings the Board gave a preliminary view in connection with transesterification and synthesis reactions, which were considered patentable. The Board however expressed doubts as to the novelty of claim 16 as granted directed to a method of hydrolysis using the immobilised lipase preparation of claim 1, because in hydrolysis immediate hydration of the lipase would make the process indistinguishable from that disclosed in document (1).
- VI. During the oral proceedings held on 2 December 1994, the Respondent submitted a main request with claims as granted, but cancelling the words "hydrolysis of fat" in claims 1 and 14, and omitting claim 16 as granted directed to a method of hydrolysis, and an auxiliary request in which the words "hydrolysis of fats and/or synthesis of fats" in claims 1 and 14 were cancelled, and both claims 16 and 17 as granted had been omitted.
- VII. In the Statement of grounds and during the oral proceedings, the Appellant questioned inventive step by developing two lines of argument. Firstly, if document (8) were to be considered as closest prior art, which disclosed the preparation of a lipase from *Candida cylindracea* immobilized on Duolite C-3, A-7, S-37, S-761 or S-762, then, although the immobilized lipase disclosed there found application in the hydrolysis of an o/w emulsion rather than in a transesterification (ie., interesterification) process, the person skilled in the art faced with a transesterification process would obviously reduce the water content of the immobilized lipase to the range prescribed in documents

(10) and (11), and thus arrive at the claimed lipase preparations. The latter documents showed that transesterification reactions were usually conducted at a water content within the range of claim 1 (2-40%). In fact, Example 7 of document (10) disclosed 8% water, while document (11) mentioned 1.1 to 30% water (page 4, line 10). Document (16) confirmed the above lower water ranges for transesterification reactions (Figure 8). Secondly, if one started from document (1) as closest prior art, which disclosed an enzyme-immobilization carrier which could be used to immobilize a wide list of enzymes, lipase being mentioned *expressis verbis*, albeit not exemplified, the only feature of claim 1 not disclosed by document (1) could be seen in the drying step which reduced the water content to 2 to 40%. For document (1) included a list of resins, among them Duolite A7, and in its examples, no non-aqueous solvents or solubility or viscosity controlling agents were used. Further, it was common practice, as exemplified by documents (10) and (11) to reduce the water content in any transesterification reaction.

The Appellant also objected to sufficiency of disclosure (Article 83 EPC) because the granted claims also covered the hydrolysis and synthesis of fats, whereas the Respondent had admitted before the USPT (see documents (9) and (12)) that the immobilized lipase according to the invention, intended for interesterification reactions, was a poor hydrolysis catalyst.

VIII. The Respondent pointed out in its counterstatement and during the oral proceedings that the invention aimed to avoid the drawbacks of the immobilized lipase preparations of the prior art, with which continuous interesterification processes could not be carried out without the addition of an organic solvent (or low alkyl

esters of fatty acids) for lowering the viscosity of the starting material, as too high a viscosity would cause a high pressure drop in the column. The invention provided immobilized lipase preparations with which transesterification could take place without the addition of organic solvents or auxiliary agents. Document (8), however, was concerned with solving a different problem, namely that of efficient hydrolysis. Documents (10) and (11) taught the addition of solvents. Therefore, the skilled person had no reason to combine features of document (8) with features of documents (10) and (11), and so the present invention would not have suggested itself to him. With a view to the Appellant's line of argument based on document (1) as closest prior art, the Respondent pointed out that if this document was taken as closest prior art, because it disclosed the potential combination of a lipase with a resin according to claim 1, no suggestion could be derived from it that such a combination would overcome the solvent problem in interesterifications with lipases.

As regards the Appellant's objection under Article 83 the Respondent cited documents (13), (14) and (15) for supporting the view that the lipase catalyst of the invention was also suited to performing hydrolysis and synthesis reactions.

IX. The Appellant requested that the decision under appeal be set aside and that the patent be revoked.

The Respondent requested that the decision under appeal be set aside and that the patent be maintained on the basis of the claims in the main request or auxiliary request respectively as submitted during the oral proceedings.

Reasons for the Decision

1. The appeal is admissible.
2. *Sufficiency of disclosure (Article 83)*

As a method of hydrolysis of fats is no longer claimed, the only issue on sufficiency of disclosure relates to the synthesis of fats. Although the description does not exemplify any synthesis reaction performed with the claimed immobilized lipase, there is a statement on page 4, lines 30 to 34 of the patent according to which a synthesis reaction occurs under the same conditions as for a transesterification reaction, i.e., by contacting the immobilized lipase according to claim 1 with a mixture of glycerol and free fatty acids in the absence of added solvents. The above statement finds experimental support in Example 1 (see Table II) of document (13). Therefore, the Board is satisfied that the requirements of Article 83 EPC are met for the claimed synthesis of fats.

3. *Novelty*

At the opposition stage it had been argued that document (8) was novelty destroying. It discloses the adsorption of 10 mg of a microbial lipase from *Candida cylindracea* dissolved in 200 µl buffer, on 0.25 g of the weak anion exchange resin Duolite (see under the heading "Material and Methods" on page 107). The Parties and the Board agree that the immobilized lipase of document (8) contains 43,47% water $[200 \times (250 + 10 + 200)^{-1} \times 100]$. Since the claims in dispute only allow an immobilized lipase with a water content of from 2 to 40%, they are novel over document (8). In fact, the novelty objection raised at the opposition stage was not maintained on appeal.

4. *The closest prior art*

As indicated during the oral proceedings, the Board considers document (5) to be the closest prior art and the most appropriate starting point. This is because document (5), unlike documents (1) and (8), is concerned specifically with transesterification reactions using a microbial lipase. The lipase of document (5) is immobilized on inert particulate carriers such as kieselguhr. With the immobilized lipase preparations of the prior art, as represented by document (5), transesterification reactions could not be performed without the addition of organic solvents, which served to control the viscosity and solubility of the reagents and had to be removed later. While using organic solvents in the preparation of fats for food products was highly questionable, because of possible contamination of the product by the solvents, the absence of organic solvents generated an unacceptably high pressure drop in the column, especially in continuous esterification processes.

5. *The problem*

On the basis of the disclosure of document (5) the objective technical problem to be solved can be seen in the provision of a lipase catalyst which avoids the risk of contamination, while yet ensuring that the pressure drop in the column is not unacceptably high. The Board is satisfied that the claimed lipase catalyst, comprising a microbial lipase immobilized on a weak anion exchange resin with a water content of from 2 to 40%, solves the above problem, since it opens up the possibility of carrying out transesterification reactions without the addition of solvents.

6. *Inventive step*

Document (5) does not itself contain any teaching that helps to solve the above problem, as the inert particulate carriers there suggested require the use of organic solvents to keep the pressure drop acceptable.

7. Document (8) is concerned with solving a quite different problem to that stated above, namely that of efficient hydrolysis. In relation to hydrolysis, this document teaches that a lipase immobilized on Duolite, a weak anion exchange resin, is inferior to other resin/immobilization methods. Even allowing for the fact that a prior art disclosure relating to hydrolytic activity may have no predictive worth as to interesterification activity, document (8) cannot be taken as recommending that Duolite be used for any purpose, let alone for interesterification. Whereas the skilled person might think it worthwhile testing a catalyst which is recommended for hydrolysis, to see if it is also good for interesterification, there seems no incentive to pick on of several hydrolysis catalysts which the comparisons in Table 1 of document (8) show give poor results for hydrolysis for the purpose of investigating its use for interesterification.

8. In the absence of any hint that it might be useful for interesterification there was no reason for the skilled person to adjust the water content of the comparative example using Duolite of document (8) to the conditions exemplified in documents (10) and (11) for interesterification, by reducing the water content so that it falls within the limits of claim 1. Nor could the Appellant provide any reason why the skilled person should reduce the water content, if for some reason despite its disclosure in document (8) being only by way of an inferior comparative example, he wanted to use it

for the purpose of hydrolysis. The expectation would be that in use it would again absorb water, so that reducing the water content would seem futile.

9. Document (1) is concerned with enzyme-immobilization on synthetic resins containing diethylaminoethyl groups. These are weak anion exchange resins. Document (1) does not mention the solvent problem arising in interesterifications with lipases, nor does it specify whether the lipase is to catalyse hydrolysis, transesterification or synthesis, and no examples relate to its use. In view of this, the fact that the examples of document (1) do not prescribe the use of solvents or solubility and viscosity controlling agents, cannot be considered as any form of hint that an acceptable pressure drop could be obtained in a column without the need to use organic solvents. Further the lipase is only one of fifty five enzymes listed in document (1), and in the context of the very large number of possible lipase and/or enzyme carriers available in the prior art, such as Celite, kieselguhr, Spherosil, urethane polymers, crossed-linked dextran, diatomaceous earth, Perlite, Duolite, kaolinite, there appears no reason why the skilled person should expect to be able to solve his problem using this resin.

10. The Board accordingly considers that no case for obviousness has been made out.

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 that the patent be maintained on the basis of the claims in the main request submitted during the oral proceedings and a description to be adapted.

The Registrar:

The Chairwoman:

L. McGarry

U. M. Kinkeldey