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Datasheet for the decision of 13 November 2012

Case Number: T 1688/10 - 3.3.09

Application Number: 01903376.0

Publication Number: 1251744

IPC: A23B 7/10, A23D 9/00, C12N 1/00, C12P 7/64, C12N 15/52

Language of the proceedings: EN

Title of invention:
Enhanced production of lipids containing polyenoic fatty acids by high density cultures of eukaryotic microbes in fermentors

Patent Proprietor:
DSM IP Assets B.V.

Opponent:
Lonza AG
Patent & Licensing Department

Headword:
-

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step - yes"

Decisions cited:
-

Catchword:
-

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Case Number: T 1688/10 - 3.3.09

D E C I S I O N
of the Technical Board of Appeal 3.3.09
of 13 November 2012

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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 21 June 2010 concerning maintenance of European patent No. 1251744 in amended form.**

Composition of the Board:

Chairman: W. Sieber
Members: J. Jardón Álvarez
K. Garnett

Summary of Facts and Submissions

I. This decision concerns the appeals filed by the patent proprietor and the opponent respectively against the interlocutory decision of the opposition division that European patent EP-B-1 251 744 in the name of MARKET BIOSCIENCES CORPORATION (now DSM IP Assets B.V.) as amended meets the requirements of the EPC.

II. The granted patent contained 25 claims, independent claims 1, 2 and 22 reading as follows:

"1. A process for producing microbial lipids comprising:

(a) conducting a fermentation of a medium comprising microorganisms, a carbon source and a limiting nutrient source and providing conditions sufficient to maintain a dissolved oxygen level of at least about 4% of saturation in said fermentation medium to increase the biomass density;
(b) subsequently providing conditions sufficient to maintain a dissolved oxygen level of about 1% or less of saturation in said fermentation medium and providing conditions sufficient to allow said microorganisms to produce said lipids; and
(c) recovering said microbial lipids,
wherein at least about 15% of said microbial lipids are polyunsaturated lipids; and
wherein a biomass density of at least 100 g/L is achieved during the fermentation."

"2. A process for enriching the polyenoic fatty acid content of a microorganism comprising fermenting said

microorganism in a growth medium having a level of dissolved oxygen of less than 1% of saturation."

"22. A heterotrophic process for producing products and microorganisms comprising culturing said microorganisms in a growth medium having a level of dissolved oxygen at less than about 1% of saturation wherein said microorganisms contain polyketide synthase genes."

Claims 3 to 21 and 23 to 25 were dependent claims.

III. Notice of opposition had been filed by Lonza AG requesting revocation of the patent in its entirety based on the grounds of Article 100(a) EPC (lack of novelty and inventive step) and Article 100(b) EPC (sufficiency of disclosure).

The documents cited during the opposition proceedings included:

D2: WO 98/03671 A1;

D5: WO 94/08467 A1;

D7: D.H. Beach *et al.*, "Environmental influences on the docosahexaenoate content of the triacylglycerols and phosphatidylcholine of a heterotrophic marine dinoflagellate, *Crypthecodinium cohnii*", *Biochimica et Biophysica Acta*, 316 (1973), pages 56-65;

D10: Third party observations on behalf of Nutrinova Nutrition Specialities GmbH dated 28 February 2005;

D13: D. Facciotti *et al.*, "Cloning and Characterization of Polyunsaturated Fatty Acids (PUFA) Genes from Marine Bacteria", Proceedings of International Symposium on Progress and Prospect of Marine Biotechnology (ISPPMB'98), 6-9 October 1998, Qingdao, China, pages 404-405;

D16: F. Chen *et al.*, "Effect of C/N ratio and aeration on the fatty acid composition of heterotrophic *Chlorella sorokiniana*", *Journal of Applied Phycology* 3 (1991), pages 203-209; and

D19: Declaration of Joseph W. Pfeifer III dated 26 March 2010.

IV. The interlocutory decision of the opposition division announced orally on 29 April 2010 and issued in writing on 21 June 2010 was based on a main request (claims as granted) and auxiliary request 1 filed by the patent proprietor during the oral proceedings.

Claim 1 of auxiliary request 1 was identical to granted claim 1. Claims 2 and 22 corresponded to granted claims 2 and 22 including further limitations with regard to the microorganism, the lipid production rate and the content of polyunsaturated lipids.

The opposition division concluded that the patent contained sufficient disclosure to enable the skilled person to carry out the invention without undue burden and thus satisfied the requirement of Article 83 EPC. However, the opposition division rejected the main request of the patent proprietor because the subject-

matter of claims 2 and 22 was anticipated by the disclosure of document D2.

As regards auxiliary request 1, the opposition division found that the subject-matter claimed in this request was novel and involved an inventive step.

V. Appeals against this decision were filed on 2 August 2010 by the patent proprietor, and on 20 August 2010 by the opponent. The respective appeal fees were paid in due time.

As the patent proprietor and the opponent are both appellant and respondent in this appeal proceedings, for simplicity the board will continue to refer to them as the patent proprietor and the opponent.

In its statement of grounds of appeal filed on 21 October 2010 the patent proprietor requested maintenance of the patent as granted (main request), in the alternative maintenance of the patent on the basis of auxiliary request 1 deemed allowable by the opposition division. The opponent requested in its statement of grounds of appeal filed on 20 October 2010 that the decision under appeal be set aside and the patent be revoked.

VI. Further submissions were filed by the patent proprietor on 1 June 2011 including auxiliary request 2.

On 22 February 2011 and on 21 November 2011 the opponent filed further submissions including a fresh document:

D20: L. Provasoli *et al.*, "Nutrition of the American Strain of *Gyrodinium cohnii*." Archiv für Mikrobiologie 42 (1962), pages 196-203.

VII. In response to the board's communication, issued on 20 March 2012 in preparation for the oral proceedings, the patent proprietor filed on 11 October 2011 a declaration by Dr. Kirk Apt in support of its arguments and further auxiliary requests, namely auxiliary request 3, and primed sets of claims (main request primed and auxiliary requests 1 to 3 primed).

VIII. In the letter dated 22 October 2012 the opponent requested that the declaration by Dr. Apt be not admitted into the proceedings. The letter dated 26 October 2012 included information concerning the deposit of the microorganism Ulkenia sp. SAM 2179 used in D2.

IX. During the oral proceedings held on 13 November 2012, the proprietor requested that the primed sets of claims be considered first, in particular the main request primed. After discussing the patentability of the subject-matter of claims 1 and 2 of the main request prime, the next request considered was, at the patent proprietor's request, auxiliary request 3 primed.

As a reaction to this discussion, the patent proprietor filed a new main request to replace all its previous requests on file. The twelve claims of this request include only one independent claim resulting from the combination of granted claims 1 and 13. This claim reads as follows:

"1. A process for producing microbial lipids comprising:

(a) conducting a fermentation of a medium comprising microorganisms, a carbon source and a limiting nutrient source and providing conditions sufficient to maintain a dissolved oxygen level of at least about 4% of saturation in said fermentation medium to increase the biomass density;

(b) subsequently providing conditions sufficient to maintain a dissolved oxygen level of about 1% or less of saturation in said fermentation medium and providing conditions sufficient to allow said microorganisms to produce said lipids; and

(c) recovering said microbial lipids,

wherein at least about 15% of said microbial lipids are polyunsaturated lipids; and

wherein a biomass density of at least 100 g/L is achieved during the fermentation;

wherein said microorganisms are order Thraustochytriales."

Claims 2 to 12 are dependent claims.

X. The arguments presented by the patent proprietor in its written submissions and at the oral proceedings insofar as they are relevant for the present decision may be summarised as follows:

– The disclosure of D5 represented the closest prior art document, which was directed to the production of microbial products with high concentration of omega-3 highly unsaturated fatty acids. The subject-matter of claim 1 differed from the closest prior art in that the fermentation was

conducted in a two-step process using a level of dissolved oxygen below 1% in the second step. The technical effect arising out of this difference was an increased production of polyunsaturated lipids, as demonstrated by the examples in the patent and confirmed by the further experimental evidence on file, namely D19 and D10.

– There was no hint in the cited documents that the formation of polyunsaturated lipids would be increased by modifying the process of D5, in particular by lowering the oxygen content in the second step. D5 itself was silent about any influence of the amount of dissolved oxygen to improve the content of polyunsaturated lipids. The achieved effect was surprising in view of the cited prior art which in fact suggested that the presence of oxygen was necessary to produce polyunsaturated fatty acids (D7, D16 and D13).

– Finally, D7 could not be used as the closest prior art as this document was an academic review published back in 1973 and actually emphasizing that the absence of oxygen resulted in more saturated fatty acids.

XI. The relevant written and oral arguments of the opponent may be summarised as follows:

– The subject-matter of all the claims lacked inventive step in view of the teaching of D7 alone. D7 disclosed a process for producing microbial lipids showing all the features of the process of claim 1 except the kind of microorganism used and

the biomass density. In fact the process disclosed in D7 was a two-step process using first a high level of dissolved oxygen and then a lower level as a result of using nitrogen. The product therein obtained contained more than 15% of polyunsaturated lipids, as could be calculated from the data in Figures 2 to 4 of D7.

– Since the microorganism *c. cohnii* investigated in D7 was a similar marine microorganism to the microorganism now required in claim 1, the technical problem to be solved by claim 1 had be seen merely as to increase the biomass density of the process of D7. The proposed solution, namely attaining a biomass density of at lest 100g/L as set out in claim 1, was a desire which could be achieved by the skilled person using his common general knowledge. Therefore, the subject-matter of claim 1 did not involve an inventive step.

– The opponent also maintained that no evidence had been provided by the patent proprietor of an increased production of polyunsaturated lipids. In the examples in the patent no control had been provided so that the alleged increase had not been demonstrated. In fact in example 7 of the patent the docosohexaenoic fatty acid (in the following, DHA) productivity was not significantly increased after reducing the amount of dissolved oxygen in the second step of the process.

XII. The appellant patent proprietor requested that the decision under appeal be set aside and the patent be maintained on the basis of claims 1 to 12 of the main

request as filed during the oral proceedings before the board.

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

Reasons for the Decision

1. The appeal is admissible.

2. Having regard to the patent proprietor's final and only request (points IX and XII above) the only issue remaining in the appeal was inventive step. No other objections were raised by the opponent against this request nor did the board see any reason to raise any objection on its own.

3. *Inventive step*

3.1 The invention as now claimed relates to a process for producing microbial lipids containing at least 15% of polyunsaturated lipids by fermentation of a medium comprising microorganisms of the order Thraustochytriales and providing, first, conditions sufficient to maintain a dissolved oxygen level of at least 4% of saturation and subsequently providing conditions to maintain a dissolved oxygen level of 1% or less of saturation.

3.2 Closest prior art

3.2.1 As indicated in the introduction of the patent specification, the production of polyenoic fatty acids

such as omega-3 and/or omega-6 polyunsaturated fatty acids in eukaryotic microorganisms is already well known. However it has been generally believed that the presence of molecular oxygen is required, i.e. aerobic conditions (see in particular paragraph [0002]).

3.2.2 In this context document D5 discloses that certain marine microalgae of the order Thraustochytriales, especially when grown at low chloride levels, produce high levels of omega-3 highly unsaturated fatty acids (see claim 1 and tables 2 and 3). The process of D5 is particularly useful in commercial production because the chloride content in the culture medium can be significantly reduced, thereby avoiding the corrosive effects of chloride on fermentation equipment (page 2, lines 17-28). D5 also recognizes the influence of the oxygen content in the production of lipids and suggests an oxygen content of less than about 40% of saturation and preferably between 5% and 40% of saturation (page 11, lines 24-27).

3.2.3 Since D5 is in the field of producing polyenoic fatty acids from microorganisms as required now in claim 1, the board considers, in agreement with the patent proprietor, that D5 represents the closest prior art document.

3.2.4 In contrast to this, the opponent relied on D7 as the closest prior art, basically because it discloses a two-step fermentation process wherein the oxygen level is reduced in the second step.

D7 discloses a process for producing microbial lipids by fermentation of a marine microorganism,

Crypthecodinium cohnii, in a medium comprising a carbon source and a limiting nutrient source. Various growing conditions are investigated in this scientific article, *inter alia* providing first a high content of oxygen followed by a restricted supply of oxygen (gassing with nitrogen: see page 59, paragraph headed "Oxygen availability"). The opponent has calculated from figures 2 and 4 that the amount of polyunsaturated lipids formed after 3 and 6 hours is about 26% and 18%, respectively, and thus above the 15% required by claim 1. Apart from the fact that D7 uses a different microorganism, the biomass density is much lower than the at least 100 g/L required in claim 1, namely 2.6 g/L, as calculated by the opponent from table 1 (0.002119×10^9 cells/l x 124 mg/10^9 cells/10 %).

In the board's judgment, the opponent's approach to assess inventive step by starting from D7 is flawed, because this document does not address the objectives of the claimed invention. D7 does not relate in any way to the problem of increasing the amount of polyunsaturated lipids. Although the amount of polyunsaturated fatty acids during the fermentation (after 3 or 6 hours) might be within the values of claim 1, the fatty acids produced at the end of the fermentation are mainly saturated fatty acids. The clear teaching of D7 is that by gassing with nitrogen, that is to say by reducing the level of dissolved oxygen in the fermentation medium, the lipid production shifts in favour of the saturated fatty acids (see D7, summary, and also page 61, lines 3-6). A document having the opposite effect to the patent in suit cannot realistically qualify as the closest prior art document, regardless of the number of technical features it might

have in common with the subject-matter of the patent. Taking it as the closest prior art document involves a hindsight approach which is to be avoided. Quite apart from that, the patent proprietor pointed out that, contrary to the opponent's assertion, the microorganism used in D7 was quite different from the one required now by claim 1. A person skilled in the art trying to optimize the production of polyenoic fatty acids from microorganisms of the order Thraustochytriales would therefore not start from a document concerned with the culturing of the microorganism *c. cohnii*.

For these reasons D7 cannot qualify as the closest prior art document.

3.3 Problem to be solved and its solution

3.3.1 According to the patent proprietor the problem underlying the patent in suit in the light of D5 is the provision of an improved process which results in an increase in the production of polyunsaturated lipids.

3.3.2 As a solution to this problem, the patent in suit proposes the process of claim 1, which is essentially characterized by controlling the dissolved oxygen level during fermentation. Thus the dissolved oxygen level is maintained above 4% during the biomass density increasing stage (step (a) of claim 1), which is then reduced below 1%, to allow the microorganisms to produce the lipids (step (b) of claim 1).

3.3.3 The experimental evidence on file shows that carrying out the fermentation as required by claim 1 enhances the production of lipids and the content of

polyunsaturated lipids. Although a direct comparison with the process of D5 has not been made, the examples in the patent show that improved production of polyunsaturated fatty acids is achieved when reducing the amount of dissolved oxygen in the fermentation.

Thus, the fermentation according to example 4 (wherein the dissolved oxygen level in the fermentation medium is reduced from 8% during the first 24 hours to 4% from the 24^{th} hour to the 40^{th} and further reduced to 0.5% from the 40^{th} hour to the end) yields higher productivity of DHA than the fermentation according to reference example 3 (wherein the dissolved oxygen level is not reduced below 1%). The average productivity (grams of DHA/L/Hr) increases from 0.288 to 0.409 and the DHA content from 16.7% to 20.6% (see Tables 2 and 4) when working with the reduced oxygen level.

In view of example 7 of the patent, the opponent doubted that the production of polyunsaturated acid actually increases. In this example a kinetic profile of a fermentation process is presented and the DHA productivity after 90 hours (0.42 g/L/hr) is slightly below the value after 36 hours (0.44 g/L/hr), the oxygen level content not having been reduced.

It may be that in example 7 no improvement in the productivity of DHA is achieved. However, this example cannot bring into question the above finding that working according to the claimed process results in an increase of the production of polyunsaturated lipids.

In fact the results in the patent are confirmed by the further evidence on file, namely D19 and D10. The

further experiments provided by the patent proprietor during the opposition proceedings, D19, show that working as required by the process of claim 1 yields a good lipid productivity and DHA productivity (see Exhibits A and B, last two entries). Moreover in D10, a third party document, a direct comparison is made between a fermentation carried out using an oxygen level of 5%, and therefore outside the scope of claim 1, and a fermentation at an oxygen level of 0.4% as required by claim 1. The results in D10 show an increase in DHA of about 17.5% (page 2, Table), confirming the results in the patent that the content of polyunsaturated lipids is increased when the amount of dissolved oxygen is decreased.

3.3.4 In view of these results, the board is satisfied that the technical problem of providing a process resulting in an increase in the production of polyunsaturated lipids has been credibly solved by the process of claim 1.

3.4 Obviousness

3.4.1 The question which remains to be decided is whether in view of the available prior art documents, it would have been obvious for the skilled person to solve this problem by the means claimed.

3.4.2 There is no hint to this solution in any of the cited documents. The document mainly relied upon by the opponent, D7, in fact teaches away from the present invention as it indicates that the use of low levels of dissolved oxygen results in a shift in favour of saturated fatty acids. In the summary of this document

it is stated that gassing with nitrogen caused a marked elevation of saturated fatty acids and a depression of unsaturated fatty acids in triacylglycerols (see also page 59 under "Oxygen availability" and page 61, first full paragraph). Thus, while indeed D7 recognizes the influence of the oxygen availability in the lipid composition, the result therein obtained is the opposite of the result in the patent in suit. The teaching of D7 does not give any hint to the process now claimed.

3.4.3 Similar considerations apply to the other documents cited in the proceedings which in fact confirm the teaching of D7 that a higher amount of dissolved oxygen was believed to be required for the formation of unsaturated lipids (see D16, page 208, left column, lines 4-8 and D13, page 405, lines 10-11).

3.4.4 Thus, the skilled person would get no incentive from D7 or from the other cited documents to reduce the level of dissolved oxygen in order to find a solution to the technical problem defined in point 3.3.1 above.

3.5 It follows from the above that the subject-matter of claim 1, and by the same token the subject-matter of dependent claims 2 to 12, involves an inventive step within the meaning of Article 56 EPC.

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 12 according to the main request as filed during the oral proceedings before the board after any necessary consequential adaptation of the description.

The Registrar The Chairman

M. Cañueto Carbajo W. Sieber