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Datasheet for the decision of 5 September 2022

Case Number: T 1424/18 - 3.3.08

Application Number: 07797800.5

Publication Number: 2024504

IPC: C12P5/00, C12P5/02, C12P7/04, C12N15/52

Language of the proceedings: EN

Title of invention:
PRODUCTION OF ISOPRENOIDS

Patent Proprietor:
Amyris, Inc.

Opponent:
Ajinomoto Co., Inc.

Headword:
Recombinant production of isoprenoids/AMYRIS

Relevant legal provisions:
EPC R. 80
EPC Art. 84, 83, 123(2), 54, 56, 87

Keyword:
Main request - requirements of the EPC complied with - (yes)

Decisions cited:
G 0001/03, T 0388/15, T 1406/14, T 0061/14, T 1811/13, T 1646/12, T 2221/10, T 0197/10, T 0593/09, T 0177/08, T 0608/07, T 0063/06, T 0292/85

Catchword:

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European Patent Office
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Beschwerdekammern

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Case Number: T 1424/18 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 5 September 2022

Appellant: (Patent Proprietor)	Amyris, Inc. 5885 Hollis Street, Suite 100 Emeryville, CA 94608 (US)
Representative:	Hayes, Emily A.L. Mewburn Ellis LLP Aurora Building Counterslip Bristol BS1 6BX (GB)
Respondent: (Opponent)	Ajinomoto Co., Inc. 15-1 Kyobashi 1-Chome Chuo-ku, Tokyo 104-8315 (JP)
Representative:	Zeman, Steven M. Grünecker Patent- und Rechtsanwälte PartG mbB Leopoldstraße 4 80802 München (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 27 March 2018 revoking European patent No. 2024504 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

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

Summary of Facts and Submissions

I. European patent No. 2 024 504 is based on European patent application No. 07797800.5, (published as WO 2007/140339 on the 6 December 2007, hereinafter "the patent application"). It was opposed on the grounds of Article 100(a) in conjunction with Articles 54, 56 and 57 EPC, and of Articles 100(b) and (c) EPC. An opposition division considered that the main request before it infringed Article 123(3) EPC, that Auxiliary request 1 did not meet the requirements of Article 83 EPC and that auxiliary request 2 infringed Article 123(2) EPC. The patent was revoked.

II. The patentee (appellant) lodged an appeal. With its statement of grounds of appeal, appellant submitted a main request and auxiliary requests 1 to 7.

III. The respondent (opponent) replied to appellant's statement of grounds of appeal.

IV. The parties were summoned to oral proceedings as requested by both parties.

V. With a letter dated 14 February 2022, without making any substantive submissions, respondent informed the board that it was not going to attend the scheduled oral proceedings.

VI. With a letter dated 1 March 2022, appellant re-filed its previous Auxiliary Request 1 as Main Request in conjunction with adapted and amended pages 3, 4, and 20 of the description and subsequently an amended page 3a with a letter dated 3 March 2022.

VII. Oral proceedings were cancelled.

VIII. Independent claim 1 according to the main request reads as follows:

"1. A method for producing an isoprenoid comprising:
(a) obtaining a plurality of bacterial or fungal host cells that comprise a mevalonate pathway for making isopentenyl pyrophosphate, wherein all of the pathway enzymes are under control of at least one heterologous transcriptional regulator and wherein one or more of the mevalonate pathway enzymes is encoded by a heterologous nucleic acid sequence;
(b) culturing the host cells in a medium comprising a reduced feed of a carbon source to improve the amount of isoprenoid produced, wherein the carbon source is maintained at a level that is suboptimal and provides for less than 90% of the maximum specific growth rate as compared to a carbon source level that would provide for a maximum specific growth rate for the host cells; and
(c) recovering the isoprenoid produced."

Claims 2 to 10 are directed to preferred embodiments of claim 1.

IX. The following documents are cited in this decision:

A1: US 5912113 (published on 15 June 1999);

A2 J. D. Newman *et al.* , Biotechnology and Bioengineering, vol.95(4), pages 684 to 691, (5 November 2006);

A3 V. JJ. Martin *et al.*, Nature biotechnology, vol. 21 (7), pages 796 to 802 (2003);

A4 D. J. Pitera *et al.*, Metabolic engineering vol. 9(2), pages 193 to 207 (2007);

A5 T. Yamanè and S. Shimizu, Advances in Biochemical Engineering/Biotechnology, vol 30, pages 147 to 194, (1984);

A6 EP 0553085 B1 (published on 28 November 1991);

A9 Declaration of Lishan Zhao, signed on 8 May 2017;

A10 Exhibit A:

A11 Exhibit B

A12 Jacques Monod, "THE GROWTH OF BACTERIAL CULTURES", pages 371 to 394, (1949);

A13 L. Yee and H.W. Blanch, BIO/TECHNOLOGY, vol. 10 (12), pages 1550 to 1556 (1992);

A15 P. Van Hoek *et al.*, Applied and environmental microbiology, vol. 64(11) pages 4226 to 4233, (1998);

A16 R.H. De Deken, Journal of general microbiology vol. 44(2), pages 149 to 156, (1966);

A17 Supplemental Declaration of Lishan Zhao, signed on 17 January 2018.

A18 Exhibit C

A22 G.W. Luli and W.R. Strohl, Applied and environmental microbiology, vol. 56(4) pages 1004 to 1111 (1990).

A26 M.A. Eiteman, and E. Altman, Trends in biotechnology, vol. 24(11) pages 530 to 536 (2006);

A28 Declaration of Yoshinori Tajima, signed on 4 December 2018.

X. The **appellant's** written submissions, insofar as relevant to the present decision, may be summarized as follows:

Rule 80 EPC and Article 84 EPC

In the decision under appeal, the "reduced feed" introduced into claim 1 was considered to be a genuine attempt to overcome a ground of opposition, which was decisive for complying with the requirements of Rule 80 EPC (see item 15.3 of the decision). It clarified and limited the feature "suboptimal" of the subsequent "wherein" clause. The definition/benchmarking of the term "suboptimal" applied to the term "reduced feed" was accordingly consistent with paragraph [0194] of the patent application (see item 20.3 of the decision).

Article 83 EPC

Appellant disputed that (i) the MSGR could not be reliably determined by the skilled person, and (ii) that the technical effect of improved isoprenoid production cannot plausibly be achieved over the entire scope of claim 1 especially at either the upper or lower end of the range of "less than 90% of the maximum

specific growth rate". As regards the ambiguous definition of "less than 90% of the maximum specific growth rate", reference was made to decisions T 61/14, T 608/07 and T 1811/13. Respondent had not provided any serious doubts substantiated by verifiable facts that the technical effect underlying the present invention could not be achieved across the entire range of "less than 90% of the maximum specific growth rate" claimed. Figures 12A and 12B of the patent provided evidence that when host cells were cultured under excess or restricted carbon source conditions, the technical effect of improved isoprenoid production was actually achieved. Figures 12A and 12B showed also that isoprenoids could be produced even in the absence of cell growth.
The inhibition occasioned by toxic products, such as acetate, generated during cell growth at the upper end of the "less than 90% maximum specific growth rate" range was reduced, as any decrease in growth rate reduced their production and accumulation, and thereby improved the cellular isoprenoid production.

Novelty (Article 54 EPC)

Novelty was not addressed in the decision. However, in the Summons to oral proceedings dated 6 June 2017, the opposition division was of the opinion that the main request was novel over A2 and A3, because none of them disclosed culturing the host cells in a medium comprising a reduced feed of a carbon source to improve the amount of isoprenoid produced (see paragraph 11.3 of the Summons). This finding applied to claim 1 of the present main request.

Document A2 described an *E. coli* strain comprising pMevT, pMBIS and pADS for the production of

amorphadiene. The *E. coli* were cultured in standard TB medium and TB with additional glycerol (see Figure 3). The OD_{600} of the engineered *E. coli* in TB medium with additional glycerol was higher than the OD_{600} of the engineered *E. coli* in standard TB medium. The *E. coli* cultured in TB medium with additional glycerol produced more amorphadiene than the *E. coli* cultured in standard TB medium. Figure 3 of document A2 demonstrated that including additional carbon increased the growth rate for the *E. coli*, but also increased amorphadiene (i.e. isoprenoid) production. This was contrary to the requirements of claim 1 reciting that culturing the host cells in a medium comprising a reduced feed of a carbon source improved the amount of isoprenoid produced.

Document A3 disclosed engineered *E. coli* that comprised pMPKPMX, pMevB, pMBI, or pMBIS for production of amorphadiene (see Figures 1 and 3). The *E. coli* were cultured in medium with increasing amounts of mevalonate. Figure 3 showed that an increase in mevalonate led to growth inhibition. It disclosed nowhere that culturing host cells in a medium comprising a reduced feed of a carbon source improved the amount of isoprenoid produced.

Document A4 did not demonstrate that culturing the host cells in a medium comprising a reduced feed of a carbon source improved the amount of isoprenoid produced. Three fermentations with different amounts of added mevalonate were performed (see Figure 2), but none of them was a culture in which a reduced feed of carbon was added. The cell growth rate for cells comprising all the MEV pathway enzymes as shown in Figure 2 was not disclosed. The cell growth reported in Figure 3

engineered with an incomplete mevalonate pathway could not remedy this deficiency.

Inventive step (Article 56 EPC)

Inventive step was not addressed in the decision. In paragraph 12.3 of the Summons to oral proceedings dated 6 June 2017, the opposition division was of the opinion, in accordance with G 1/03 (Reasons 2.5.2), that the technical effect of improved isoprenoid production, recited in claim 1, was not to be considered under Article 56 EPC.

Document A2 represented the closest prior art for the subject-matter of claim 1. It was concerned with the same purpose, i.e. to increase isoprenoid production by varying the provision of a carbon source.
The difference between claim 1 and document A2 was that claim 1 required culturing the host cells in a medium comprising a reduced feed of a carbon source to improve the amount of isoprenoid produced, wherein the carbon source was maintained at a level that is suboptimal and provides for less than about 90% of the maximum specific growth rate as compared to a carbon source level that would provide for a maximum specific growth rate for the host cells.

Culturing the host cells in a medium comprising such a reduced feed of a carbon source, resulted in an improved isoprenoid production.

This effect was demonstrated in Figure 12B and shown to be solved in Figures 12A to 12C.

The key issue was whether or not the proposed solution of reducing carbon feed to decrease growth rate and

increase isoprenoid production was obvious in view of the cited prior art.

Document A3 taught away from reducing the carbon feed in order to improve isoprenoid production, while document A1 referred to L-lysine production instead of isoprenoid production, document A4 neither disclosed nor suggested to use a reduced feed of carbon source, document A5 referred to repression of enzymes involved in catabolic pathways. The repression was caused by an increased intracellular presence of ATP. There was however no teaching or suggestion that mevalonate pathway enzymes would be similarly affected by catabolite repression due to an increase of glucose. Document A6 related to a method for the efficient fermentation of heterotrophic algae that increased the production of xanthophyll (a carotenoid compound). There was no teaching or suggestion in A6 that lowering the level of a carbon source would increase carotenoid in any other particular cell type, let alone increase isoprenoid production in the engineered mevalonate pathway expressing *E. coli* cells.

Document A13 related to recombinant product yields specifically for proteins in cells in high cell density fed batch cultures using different carbon-limited feeding strategies. The method neither disclosed nor suggested that it was capable of improving isoprenoids production as well.

XI. The **respondent's** submissions, insofar as relevant to the present decision, may be summarized as follows:

Rule 80 EPC and Article 84 EPC

The "reduced feed" introduced in claim 1 of the main request was neither admissible under Rule 80 EPC nor allowable under Article 84 EPC.

If the amendment reiterated the sense of the granted claims in different words, without materially changing that scope, then it could not be occasioned by a ground of opposition as required by Rule 80 EPC (see decisions T 759/10, item 7.2.3; T 1275/05, item 2). An amendment restating the original language of the claim was redundant and unconcise, thereby offending the requirement of Article 84 EPC. If the term "reduced feed" meant something else it lacked clarity under Article 84 EPC. It was relative and there was no reference feed with regard to which the feed had to be reduced. It was impossible for the skilled person to determine whether it would carry out the culturing step or not and thus fell within the scope of claim 1.

Article 123(2) EPC

The term "reduced feed" introduced into claim 1 step (b) had no basis in the patent application.

First, there was no basis in paragraph [0194] of the patent application for a "reduced feed" in claim 1 (b) characterizing the "carbon source" because the following clause in claim 1 (b) related to a "level" of the "carbon source".

Second, the "reduced feed of carbon source relative to the carbon feed which would provide for the maximum specific growth rate", mentioned in paragraph [0194] of the patent application, was omitted in claim 1. The reduced feed in claim 1(b) was only required to improve the amount of isoprenoid produced.

Third, even if "lowering the carbon source feed rate to a microorganism can improve the amount of isoprenoid produced in the fermentation" (see paragraph [0194] of the patent application), the term "rate" was omitted in claim 1(b), referring to "a reduced feed of a carbon source to improve the amount of isoprenoid produced". The "reduced feed" was taken out of its context and generalized to encompass any carbon source concentration below a certain threshold (see [00193] to [00195] of the patent application). The patent application provided no basis for such a method step.

Article 83 EPC

The following objections were raised:

(i) the MSGR could not be reliably determined and reproduced by the skilled person,

(ii) the technical effect of improved isoprenoid production could not plausibly be achieved over the entire scope of the method of claim 1,

(iii) the skilled person would not be able to reproduce all embodiments falling within the scope of the claim without undue burden,

(iv) serious doubts supported by evidence (the patent, documents A12 and A13) shifted the burden of proof to the proprietor to establish that the invention was sufficiently disclosed, and

(v) the method step (c) could not remedy the insufficient disclosure of preceding method steps.

Thus, first the MSGR could not be reliably determined by the skilled person, as several methods of determining MSGR leading to different values were described in the patent.

The carbon source concentration in the cell culture medium of the claimed method was defined by means of its effect on the growth rate of the host cell, which in turn was defined relative to a maximum specific growth rate (MSGR). Thus, the patent was required to disclose all relevant aspects relating to the determination of the MSGR such that a skilled person could reliably reproduce the method according to claim 1.

The MSGR had to be measured when the conditions under investigation (e.g., a substrate level or temperature) support the fastest initial growth rate (see patent paragraph [0142]). The initial growth was understood as the so-called lag phase followed by an exponential growth phase. The MSGR could also be determined at later stages of the fermentation by taking into account the appropriate variables (see paragraph [0145] of the patent). Both paragraphs mentioned contradictory time points at which MSGR had to be measured. The patent provided no explanation how the terms "later stage", "variables" had to be understood and how the variables had to be modified. On the contrary, at least three sets of instructions for measuring MSGR were proposed, resulting in different MSGR values. As a result no consistent results could be achieved (see decision T 225/93). Neither document A9, item 7, nor the focus on

one disclosure while ignoring others in the patent, were sufficient to determine/re-interpret how the term MSGR had to be understood.

Thus, the MSGR was so ill-defined in the patent, that the skilled person was not able, on the basis of the disclosure as a whole and using his common general knowledge, to identify without undue burden the technical measures (i.e. selection of the suitable time point of measuring the MSGR, selection of the suitable variables, i.e. parameters, affecting the MSGR) necessary to solve the problem underlying the patent (see decision T 815/07, item 6 and Case Law of the Boards of Appeal of the European Patent Office, 9th Edition July 2019; II.C.5.5, page 359, decision T 593/09). The findings in decisions T 815/07 and T 593/09 were not contradicted by the findings in decisions T 61/14, T 608/07 and T 1811/13.

Due to the presence of an ill-defined parameter in the disclosure of the patent, the claimed invention was insufficiently disclosed within the meaning of Article 83 EPC, and as a consequence of this insufficiency, claims defined by said parameter, i.e. MSGR, lacked also clarity under Article 84 EPC, as the exact scope of the claim could also not be established. The ambiguity of the parameter MSGR was not derived from the claim language per se, but existed because the patent disclosure as a whole prevented the skilled person from reliably, that is reproducibly, putting the invention into practice and solving the problem the patent purported to solve.

Secondly, based on the patent itself as well as documents A12 and A13, the technical effect could not

be plausibly achieved over the whole scope of claim 1 without undue burden, i.e. for all reduced carbon feed. Since the MSGR parameter was ill-defined and could not be reliably determined, a weak presumption of validity of how to carry out the invention existed. In cases where only a weak presumption of validity existed, it was established case law that serious doubts raised in the form of comprehensible and plausible arguments were sufficient to deny sufficiency of disclosure.
Thus, by plausibly arguing that the technical effect could not be plausibly achieved for all reduced carbon feed concentrations over the whole scope of the claim without undue burden, serious doubts substantiated by verifiable facts in the patent itself and in documents A12 and A13 had been raised that the invention is sufficiently disclosed (Article 83 EPC). The burden of proof was shifted to the proprietor.

Thirdly, excess carbon source concentrations during fermentation were known to generate poisonous acetate and to reduce the product formation yield. This phenomenon was known (see documents A12, A13, A22, A28) and shown in Fig. 12D of the patent, especially run "050608-1" and paragraphs [0160] and [0242]. The fermentation run "050608-1" was performed using excess of carbon, i.e. glucose negatively affecting the yield of Amorpha-4, 11-diene synthase (AMD) - an isoprenoid in the sense of the invention.

The carbon source level in claim 1 (b) was defined as any concentration that would provide for less than 90% of the MSGR, thereby including any concentration which provides for 0% of the MSGR. At this carbon source concentration, the cells would not proliferate and would not make an improved amount of isoprenoids but would be starving and be close to death.

Example 12 of the patent was the only example using the method according to claim 1. The carbon source concentration of about 45% of the MSGR enabled the production of isoprenoids, but the skilled person was unable to determine which upper and lower carbon source concentration in the fermentation medium were still capable of producing isoprenoids. Even if Example 12 demonstrated that an extremely low carbon source concentration during culturing "060403-3" allowed the production of isoprenoids while avoiding the accumulation of toxic by-products, e.g. acetate. This experiment lacked a control and thus could not be properly interpreted with respect to any carbon source concentration that would provide for a growth relative to the MSGR corresponding to the conditions of "060403-3".

The excess carbon condition "050608-1" was an arbitrary condition that comprised a highly fluctuating amount of carbon source concentration (see Fig. 12C of the patent), which were nowhere indicated to serve as control condition, let alone set the "MSGR" condition according to which, i.e. the carbon source concentration had to be determined and the carbon source concentration supporting the fastest initial growth rate with respect to which the host cells must have a growth of less than 90%. Even if "050608-1" corresponded to the MSGR, the restricted condition "050629-1" had a growth rate of approximately 49% of the MSGR of "050608-1" which did not allow a speculative extrapolation over the entire range of 0-90% MSGR.
Cells cultured at close to 0% MSGR would not produce an improved amount of isoprenoid, but would be starved and close to cell death. Without any source of carbon, no

carbon would be available for making any isoprenoid at all; let alone an improved amount thereof.
Cells cultured at a carbon concentration providing for 90% MSGR accumulated acetate, which was toxic to the cells. Thus, due to inconsistencies and methodological errors, the patent was not suitable to make the improved isoprenoid production plausible by reducing the concentration of the carbon source to 0 to 90 % of the MSGR.

The wording of claim 1 (b) required that the reduced feed of a carbon source was maintained, suggesting that the carbon source concentration in the medium of the method of the invention had to be lower than the carbon source concentration in the MSGR reference culture at all times. The minimum threshold of 5 g/l glucose in run "050608-1" was nonetheless exceeded in "050629-1", reaching a glucose concentration of over 15 g/l (see patent Fig. 12C). For this reason, the fermentation run "050629-1 " represented experimental condition falling outside the scope of the claims, because the carbon source concentration at times exceeded the MSGR carbon source concentration.

Fourthly, the non-working embodiments introduced by steps a) and b) constituted separate limitations of the method according to claim 1, which had to be sufficiently disclosed. They were essential to the sufficiency of disclosure and could not be overcome by a subsequent terminal step (c).

Novelty (Article 54 EPC)

The decision under appeal did not have to address the grounds of novelty and inventive step raised in opposition. The finding of the opposition division, in

its preliminary opinion, and of the appellant were nonetheless not shared.

Claim 1(b) related to a step of culturing cells under conditions where the carbon source was restricted so that cells did not grow at maximum speed to allow an improved isoprenoid production. Depletion of carbon source during fermentation was a common phenomenon. The cells grown in batch-fed system were known to deplete the nutrients of the fermentation medium, including the carbon source, resulting in a reduction in cell growth rate. In view of this fact, any and all cell culturing systems that utilize a batch fed system for culturing a host cell that comprises the MEV pathway as claimed will fall within claim 1.

Document A2 related to the production of Amorpha-4,11-diene (AMD) - an isoprenoid - in an *E. coli* host cell. The *E. coli* strain W3110 contained plasmids pMevT, pMBIS, and pADS, encoding the entire MEV pathway for making isopentenyl pyrophosphate (IPP) wherein all of the pathway enzymes were under the control of at least one heterologous transcriptional regulator (see page 685; Table I and Figure legend of Fig. 3). The host cells were cultured in TB medium, or in TB medium supplemented with glycerol (TBG) once the cells entered the stationary phase. The TBG medium comprised 1% glycerol as a carbon source. The AMD was recovered (see page 685, col.2, last paragraph).

Document A3 disclosed a method for producing isoprenoids, wherein a plurality of bacterial host cells were cultured. The host cells comprised a MEV pathway for making IPP, wherein all of the pathway enzymes are under control of at least one heterologous transcriptional regulator (see abstract; page 798,

bottom of left column, Figures 1 and 2). In addition to the MBIS operon, *E. coli* DH10B expressed the MevT operon (see page 797, middle of right column and Figure 1). The host cells were cultured in a medium comprising a carbon source in the form of mevalonate (see page 798, bottom of right column). The AMD was recovered in an organic layer (see page 801, col.2, first paragraph).

Document A4 disclosed a method of improved isoprenoid production comprising a bacterial host cell comprising a MEV pathway for making IPP, wherein all of the pathway enzymes are under control of at least one heterologous transcriptional regulator (see Figures 1 and 2). The host cells were grown in C medium containing 3.4% glycerol (a carbon source). The AMD was recovered in an organic layer (see page 196, col.2, last paragraph).

Although documents A2 to A4 did not explicitly disclose the step of culturing the host cells in a medium comprising a reduced feed of a carbon source concentration provides for a 0-90% MSGR, carrying out the methods of documents A2 to A4 would necessarily have led to a result falling within the terms of claim 1.

Inventive step (Article 56 EPC)

Document A2 represented the closest prior art for the subject-matter of claim 1.

It disclosed a method of high-level isoprenoid production by expressing the entire MEV pathway in *E. coli*, and further indicated that cell culture media with balanced nutrients, and optimal carbon delivery

might affect isoprenoid yield (see Title, abstract, page 690, right column, end of first full paragraph).

The difference between the claimed method and the method described in document A2 consisted in the step of culturing the cells in a reduced feed of carbon source so that it was maintained at a level that provided for less than 90% MSGR.

The technical effect associated with this difference was that the production of isoprenoid was improved. Doubts about this effect recited in claim 1 were dealt with under Article 83 EPC.

Thus, the objective technical problem was formulated as the provision of a method for improved isoprenoid production.

Document A2 explicitly underlined the importance of balancing nutrients (e.g. carbon source) as promising starting points for optimizing and increasing product production (see page 690, right column, middle of the page). It provided thus a motivation to the skilled person faced with the above technical problem to modify the conditions used in document A2 in the hope of solving said problem. The skilled person knew also, from its common general knowledge, that in a medium comprising an excess glucose under aerobic conditions the crabtree effect would inhibit recombinant protein production (see also document A13).

The solution to this technical problem was to grow cells a little slower than possible (i.e. less than the MSGR) by adjusting the concentration of carbon source in the culture medium.

The carbon source concentration used in document A2 was not in the range of the excess carbon source concentration that would entail detrimental effects, e.g. acetate formation, high growth rate and low yield. A slight increase in the concentration of the carbon source would therefore result in an increased isoprenoid production.

Starting from document A2 the skilled person trying to solve the technical problem would have used a carbon source concentration (TB or TBG) and would have modulated the optimal carbon delivery and growth rate (see page 690, col.2, lines 24 to 28). The reduction of a carbon feed into the medium in document A2, which was well below the concentration required for MSGR, would maintain the carbon source concentration at a level in the medium that would provide for less than 90% of the MSGR as compared to a carbon source level that would for the MSGR.

Alternatively, the method of claim 1 lacked an inventive step over the content of document A2 in combination with any of the content of document A1, A3 to A6.

Document A1 related to a method for cultivating yeast or bacteria in a culture medium , wherein the carbon source concentration in the culture medium was maintained at a constant low level. Documents A2 and A1 disclosed methods of producing metabolic compounds in bacterial host cells. The skilled person faced with the above technical problem was therefore motivated to combine the teaching of documents A2 and A1.

Document A5 related to fed-batch techniques and microbial processes. An excess of carbon source in the

culture medium of microbiological cells resulted in increased intracellular ATP accumulation leading to the repression of biosynthetic enzymes. A powerful method of overcoming this catabolite repression was a fed-batch culture in which the glucose concentration in the culture medium was kept low (see sections 4.5 and 2.4). Fed-batch culture with restricted carbon levels in the production of various metabolites was broadly applicable (see page 156 to 157 sections 4.2 to 4.5). It obviously extended to isoprenoids.

Document A6 related to a method of culturing an algae for the production of an isoprenoid (xanthophyll), wherein the production of the isoprenoid was optimized by continuously diluting the growth medium with fresh growth medium comprising a growth-limiting amount of carbon source. It explicitly taught that the dilution of the growth-limiting amount of carbon source should result in a growth rate that is less than the MSGR and provided preferred ranges of about 25 - 95% of the MSGR (see paragraphs [0027],[0029]).

In conclusion, the method of claim 1 lacked an inventive step over the content of document A2 alone or in combination with the common general knowledge or any of the documents A1, A3 to A6.

XII. The appellant requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request filed with the letter dated 1 March 2022, in conjunction with amended pages 3, 3a, 4, and 20 of the description.

XIII. Respondent requested that the appeal be dismissed.

Reasons for the Decision

Main request (claims 1 to 10)
Rule 80 EPC and Article 84 EPC

1. The respondent contended that the amendment "reduced feed" of a carbon source, introduced into claim 1 only intended to restate that the carbon source was maintained at a suboptimal level. This amendment was not occasioned by a ground of opposition as required by Rule 80 EPC, as it materially did not change the scope of granted claim 1.

1.1 If the "reduced feed" introduced a true limitation, in that it defined something else or how maintenance of the carbon source level was effected, then the amendment did not determine with regard to which carbon source reference value it had to be reduced. Thus, the amendment "reduced feed" was either not occasioned by a ground of opposition and contravened Rule 80 EPC or it led to a clarity problem. Either way, the amendment was inadmissible.

2. In the decision under appeal, the introduction of "reduced feed" into claim 1 was considered to be a genuine attempt to overcome a ground of opposition, which was decisive for complying with the requirements of Rule 80 EPC (see item 15.3 of the decision). The amendment clarified and limited the feature "suboptimal" of the subsequent "wherein" clause. The definition of the term "suboptimal" equally applied to the term "reduced feed" and was consistent with paragraph [0194] of the patent application (see item 20.3 of the decision).

3. The board considers that the term "reduced feed" introduced into claim 1 limits and defines the

"suboptimal" carbon source level feature against which an objection under Article 83 EPC had been raised (see notice of opposition, item 4.3). For this reason alone, its introduction complies with Rule 80 EPC.

3.1.1 This amendment specifies that the "suboptimal" condition is achieved by applying a "reduced feed" and restricts this condition by excluding excess feed. The amendment therefore also complies with the requirements of conciseness under Article 84 EPC.

3.1.2 The "reduced feed" of a carbon source is defined and limited by the subsequent "wherein" clause which stipulates that the carbon source is maintained at a level that is suboptimal and provides for less than 90% of the maximum specific growth rate as compared to a carbon source level allowing a maximal specific growth rate. Thus, any feed of carbon source relative to the carbon feed which would provide for the maximum specific growth rate is "reduced". Claim 1 is clear.

Article 123(2) EPC

4. The respondent argued that the term "reduced feed" introduced into claim 1 step (b) had no basis in the patent application.

There was no basis in the patent application for a "reduced feed" characterizing the "carbon source" in claim 1 (b) which related to a "level" of the "carbon source" in the subsequent clause of claim 1(b).

The "reduced feed of carbon source" according to claim 1 was only required to improve the amount of isoprenoid produced, but was not mentioned to be reduced "relative to the carbon feed which would provide for the maximum

specific growth rate" (see paragraph [0194] of the patent application).

Even if "lowering the carbon source feed rate to a microorganism can improve the amount of isoprenoid produced in the fermentation", said rate was omitted in claim 1(b). The "reduced feed" was in consequence generalized to encompass any carbon source concentration below a certain threshold (see paragraphs [00193] to [00195] of the patent application). The patent application provided no basis for such a method step.

5. The board considers that the patent application relates to a method for producing an improved amount of isoprenoids via optimal redirection of microbial metabolism (see paragraph [0006]). This is achieved by culturing the host cells in a medium, the culturing comprising maintaining the carbon source at a level that is suboptimal and provides for less than 90% of the maximum specific growth rate (see paragraphs [0010],[0186] and [0194] of the patent application and claim 20). The patent application mentions that "[t]he host cells are cultured in a fermentation medium comprises [sic] a carbon source present in an amount that is lower than that which would provide for a maximum specific growth rate" (see patent application paragraph [0186]). It is the amount of carbon source in the medium that has to be lower than the one providing for a maximum specific growth rate not the carbon source feed rate. This view is confirmed in the patent application, which uses the term "reduced feed" and not "reduced feed rate" (see paragraph [00194], first sentence).

5.1 Hence, the level of carbon source is equal to the total amount of carbon source added to the medium (feed), whilst the medium in step (b) comprises a reduced feed of carbon source which is further maintained, as introduced by the term "wherein", at a level (i.e. concentration) that is suboptimal and provides for less than 90% of the maximum specific growth rate as compared to a carbon source level that would provide for a maximum specific growth rate for the host cells.

5.2 Since in one aspect of the invention the method of producing an isoprenoid, especially high yield of isoprenoid compounds, involves the steps of culturing the host cells in a medium under conditions that are suboptimal as compared to conditions that would provide for a maximum specific growth rate for the host cells (see page 23, paragraph [00172]), and said culturing the host cells in a medium comprises a reduced feed of a carbon source (i.e. amount) which is explicitly maintained at a level that is suboptimal and provides for less than 90% of the maximum specific growth rate (see paragraphs [00186] and [00194]), the method of claim 1, in particular step (b), complies with the requirements of Article 123(2) EPC.

Article 83 EPC

6. The findings on sufficiency of disclosure in section 21.3 of the decision under appeal were contested by the appellant.

7. The opposition division concluded that establishing the MSGR was not possible without undue burden because the patent was only vague on when and how to measure it. Moreover it considered it implausible that the claimed

improvement was achievable across the whole scope of the claim.

8. The respondent argued essentially that

(i) the MSGR could not be reliably determined by the skilled person,
(ii) the technical effect of improved isoprenoid production could not plausibly be achieved over the entire scope of claim 1,
(iii) the skilled person would not be able to reproduce all embodiments falling within the scope of the claim without undue burden, and
(iv) a last method step (c) could not remedy the insufficient disclosure of preceding method steps.

The maximum specific growth rate (MSGR)

9. The respondent contended that (i) the MSGR could not be reliably determined by the skilled person, as several methods of determining MSGR leading to different values were described in the patent.

10. The board considers that the term "maximum specific growth rate" (MSGR) is a standard term known in the art. This term need not be re-interpreted in the light of the description, in a way different from how it would be understood by the person skilled in the art (see e.g. decision T 2221/10, point 33 of the reasons and decision T 197/10, headnote). No general requirement can be derived from Article 69(1) EPC that claims are to be interpreted with the help of the description (see decision T 1646/12, point 2.1), and from the second sentence of Article 69(1) EPC that an

unambiguous and generally accepted definition of a term figuring in the claims should be superseded by a different definition found in the description (see decision T 177/08, item 3.3 second paragraph of the reasons).

10.1 Claim 1(b) requires culturing the host cells in a medium comprising

(i) a reduced feed of a carbon source,

(ii) wherein the carbon source is maintained at a level that is suboptimal and provides for less than 90% of the maximum specific growth rate as compared to a carbon source level that would provide for a maximum specific growth rate for the host cells.

10.2 The maximum specific growth rate for cells in a culture medium relates to the effect of substrate concentration on growth rate. The MSGR represents for example the maximal slope obtainable on a cell growth curve on a plot representing the cell biomass concentration or optical density in function of the fermentation time.

10.3 Even if the skilled person turns to the description to understand its technical meaning, it will not come to a different conclusion. A definition for this term is provided in paragraphs [0142] to [0145] of the patent.

Although several time points for determining the MSGR are proposed in paragraph [0145], they are all options introduced by words "often" or "can".
"A theoretical treatment of the relationship between growth rate in culture is well known to those skilled in the art, and is referred to as the Monod equation. [...]. In this theoretical treatment, the maximum specific rate is an asymptotic limit that is never

reached until an infinite level of substrate is reached. In practice, however, the maximum specific growth rate can be considered as being obtained when the conditions under investigation (e.g., a substrate level or temperature) support the fastest initial growth rate. For instance, in a fed-batch reactor, the initial condition where the nutrients are supplied in excess is treated as the conditions for the maximum growth rate." (see paragraph [0142], emphasis added).

10.3.1 Despite the expression "fastest initial growth rate", used in the description in association with the term MSGR, the skilled person would not be motivated to re-interpret the term MSGR, having a well-recognised meaning in the art, in the light of the description.

Even if the skilled person turned nonetheless to the description, the term "initial" could not prima facie refer to the "lag phase", a period of time in which cells are adjusting to their environment. The cell growth rate in this phase is close to zero and increases only during a following acceleration phase.

The fastest (superlative) growth rate is commonly observed during the exponential phase, after which cell growth slows down and stops in a deceleration and a saturation phase respectively. The fastest initial growth rate amounts to the cell growth rate observed when the conditions under investigation (e.g., a substrate level or temperature) support the fastest growth rate, e.g. in a fed-batch reactor where the nutrients are supplied in excess. The term "initial" defines the fastest growth rate when the cell metabolic activity has not yet significantly modified the initial culture conditions e.g. during a multi or bi-phasic cell growth.

10.4 Although other methods for determining the MSGR are suggested in the description, at least one method allows to determine the MSGR of host cells when using a culture medium and conditions supporting a maximum specific growth rate for the host cells. Besides, there is no evidence that the other methods for determining the MSGR mentioned in the description lead to different and inconsistent MSGR values, differences which are so significant that the skilled person is deprived of the promise of the invention due to this ambiguity (see decisions T 608/07, item 2.5.2; T 593/09, catchword; T 61/14; T 1811/13, point 4 of the decision).

The MSGR may be obtained from the experimental data of Example 12 and Figure 12 of the patent. How the MSGR is determined and calculated using methods known to the skilled person is elaborated and detailed in declaration A9, section 7, exhibits A and B (A10 and A11), and declaration A17, section 10, exhibit C (A18).

Standard of proof and burden of proof

11. The respondent argued that decision T 63/06 established that while the initial burden of proof generally lay upon an opponent to establish insufficiency of disclosure, when the patent failed to give any information of how a feature of the invention, i.e. MSGR parameter, could be put into practice, only a weak presumption of validity existed that the invention was sufficiently disclosed.

11.1.1 In such a case, an opponent could discharge their burden of proof by plausibly arguing that common general knowledge would not enable the skilled person to put this feature into practice thereby placing the

burden of proof on the patent proprietor for any assertion to the contrary.

11.2 Appellant alleged that no serious doubts substantiated by verifiable facts in accordance with T 19/90 were raised to support the contention that the skilled person would not be able to obtain the technical effect of improved isoprenoid production across the entire claimed scope.

11.3 In the board's view, decision T 63/06 stipulates that after the grant of the patent, i.e. after the end of the examination proceedings, a legal presumption exists that the patent meets the requirements of the EPC. The weight of arguments and evidence required to rebut this presumption depends on its strength. When the patent does not give any information of how a feature of the invention can be put into practice, only a weak presumption exists that the invention is sufficiently disclosed (see Reasons 3.3). In such a case, as also argued by the respondent, the opponent can discharge his burden by plausibly arguing that common general knowledge would not enable the skilled person to put this feature into practice.

11.3.1 This is however not the case here. The method for producing isoprenoids in Example 12 and illustrated in Figures 12 A to D of the patent provides evidence that the invention can be put into practice (see in particular item 10.4 above). Thus, in the absence of verifiable facts, the respondent's comprehensible and plausible arguments are not sufficient to rebut the strong presumption of validity of the patent established by its Example 12.

Technical effect recited in claim not plausibly achieved

12. The opposition division in the decision under appeal and the respondent argued that a technical effect, such as the improved production of isoprenoid, had to be plausibly achievable over the entire ambit of claim 1, i.e. culturing with carbon source concentrations that provide for any growth rate from 0% to just below 90% of the MSGR (see decision G 1/03 point 2.5.2 of the reasons). The carbon source concentration in culture medium should neither be too high nor too low.

12.1 The respondent contended that an excess of carbon source during fermentation generated poisonous metabolites which prevented an improved production of isoprenoid. The addition of a feed for maintaining a carbon source level providing for 0% of the MSGR included cell cultures that did not proliferate and were close to death. Even if Example 12 of the patent maintained a carbon source level providing for 45% of the MSGR, it provided no examples showing whether upper or lower carbon source concentrations were still capable of enhancing isoprenoid production. Besides, the wording of claim 1 (b) required that the reduced feed of a carbon source was maintained, suggesting that the carbon source concentration in the medium of the method of the invention had to be lower than the carbon source concentration in the MSGR reference culture at all times.

12.2 The board considers that if a technical effect is recited in the claim an alleged lack of reproducibility of the claimed invention needs to be assessed under sufficiency of disclosure (see decision G 01/03, OJ 2004, 413, item 2.5.2 of the reasons). Since the aim of the method of claim 1 is to improve the amount of isoprenoid produced, which is allegedly not

reproducible over the whole ambit of the claim, a lack of sufficiency of disclosure may arise. The allegedly non-working embodiments of the method of claim 1 include the culturing of host cells in a medium comprising a reduced feed of a carbon source [...] wherein the carbon source is maintained at a level that is suboptimal and provides for either 0% or just below 90% of the maximum specific growth rate as compared to a carbon source level that would provide for a maximum specific growth rate for the host cells.

12.2.1 The cell growth curve of the fermentation run "050608-1" in Example 12 of the patent, comprising an excess carbon source, is the only fermentation where the cell growth rate may reach a maximum (MSGR) during its exponential phase, as its carbon source is not restricted. Figures 12 A and B of the patent provide evidence that culturing host cells under carbon-restricted conditions, "050629-1", provides for less than 90% of the maximum specific growth rate as compared to a carbon source that would provide for the maximum specific growth rate "050608-1", while Figure 12B of the patent demonstrates that isoprenoid production is higher under the carbon restricted condition "050629-1" compared to the excess carbon condition "050608-1".

There is no evidence that culturing host cells in a medium comprising a carbon source which is maintained at a level that provides for a cell specific growth rate just below 90% of the MSGR will not improve the production of isoprenoid compared to conditions providing for a MSGR for the host cells. Nor is there any evidence that isoprenoids cannot be produced and improved during the stationary phase of cell growth when the specific cell growth amounts to 0% of the

maximum specific growth rate (see "050608-1" and "050629-1"; time span from 50 to 70 hours in Figures 12 A and B of the patent). Thus, an improvement in isoprenoid production may still be seen at 0% of the maximum specific growth rate.

12.3 The board considers that claim 1 step (b) requires the step of culturing the host cells in a medium comprising a reduced feed of a carbon source, i.e. a reduced amount of a carbon source in [g], and wherein the carbon source is maintained at a level [g/l] that is suboptimal and provides for less than 90% of the MSGR as compared to a carbon source level that would provide for a maximum specific growth rate for the host cells, which level must be maintained at all times during the cell culture step, regardless of the culture phase.

Even if the carbon source minimum threshold of 5 g/l glucose in "050608-1" is exceeded in "050629-1", which may result in a glucose concentration of over 15 g/l, this is not excluded from the wording of claim 1 as the method only requires that the carbon source level in the medium is maintained at a level at which the host cell specific growth remains below the 90% of the MSGR, while the reduced feed in [g], and not its level [g/l], must be compared to the feed added in a medium which provides for a MSGR at any corresponding time during the fermentation process.

12.4 The respondent contended that the non-working embodiments introduced by steps a) and b) constituted separate limitations of the method according to claim 1, each of which had to be sufficiently disclosed. They were essential to the sufficiency of disclosure and could not be overcome by a subsequent terminal step (c). The facts underlying decision T 292/85 did not

align with the present situation. Thus, the standard for sufficiency of T 292/85 was not applicable.

12.5 The board agrees with the decision under appeal, item 21.3, second paragraph, that step c) cannot remedy a lack of insufficiency arising against an independent step of the method claimed, as the disclosure must be sufficient to put into practice essentially all parts of a claim without undue burden.

12.6 However, as stated above, the board considers that the skilled person can perform step b) without undue burden. Thus, the board concludes that the requirements of Article 83 EPC are met. The same conclusion applies to claims 2 to 10 dependent thereon.

Priority (Article 87 EPC)

13. In its communication sent in preparation of oral proceedings, the opposition division considered that the auxiliary request, corresponding to the present main request, could not validly claim priority rights from the earliest priority document US 60/808,989 because it did not disclose step (b) of claim 1.

13.1 The finding on priority in the board's communication sent to the parties during appeal proceedings was that the subject-matter of claim 1 does not enjoy priority rights from the earliest claimed priority application for the same reason found by the opposition division (see item 14). This was not contested. Hence, the board sees no reason to deviate from this finding.

Novelty (Article 54 EPC)

14. As a priority right cannot be validly claimed from the first priority application, documents A2 and A4 published before 18 December 2006 represent prior art under Article 54(2) EPC.

14.1 The decision under appeal did not have to address the objections of lack of novelty raised in opposition.

14.2 Respondent did nonetheless not share the provisional finding of the opposition division and of the appellant that the features in the method of claim 1 of "... culturing the host cells in a medium comprising a reduced feed of a carbon source to improve the amount of isoprenoid produced, ...", resulted in a higher production of the isoprenoid for the cell culture showing a lower growth rate/OD-value and vice versa, were neither disclosed in documents A2 and A3. Document A2 disclosed that cell cultures with a higher OD-value showed a higher production of isoprenoid, whereas the culture with a lower OD-value showed a lower production of isoprenoid (see Figure 3), whereas document A3 disclosed OD-value of *E. coli* DH10B harboring either the pBBR1MCS-3 (empty plasmid control), pMKPMK, pMevB, pMBI or pMBIS plasmids expressing the various mevalonate operons described in Figure 1 in media supplemented with increasing concentrations of exogenous mevalonate.

14.2.1 Claim 1(b) related to a step of culturing cell under conditions where the carbon source was restricted so that cells did not grow at maximum speed to allow an improved isoprenoid production. Depletion of carbon source during fermentation was a common phenomenon. The cells grown in batch-fed system were known to deplete

the nutrients of the fermentation medium, including the carbon source, resulting in a reduction in cell growth rate. In view of this fact, any and all cell culturing systems that utilize a batch fed system for culturing a host cell that comprises the MEV pathway as claimed would fall within the scope of claim 1.

14.3 Documents A2 to A4 relate to the production of Amorphadiene (AMD), an isoprenoid, in *E. coli* host cells. The bacterial host cells comprised a mevalonate (MEV) pathway for making isopentenyl pyrophosphate (IPP), wherein all of the pathway enzymes were under the control of at least one heterologous transcriptional regulator.

14.3.1 In document A2, the host cells were cultured in TB medium, or in TB medium supplemented with glycerol (TBG) once the cells entered the stationary phase. The TBG medium comprised 1% glycerol (a carbon source).

14.3.2 In document A3 genetically engineered *E. coli* comprising plasmids pMPKPMX, pMevB, pMBI, or pMBIS were cultured for production of amorphadiene which was recovered (see Figures 1 and 3). The *E. coli* were cultured in medium supplemented with increasing amounts of mevalonate (a carbon source).

14.3.3 In document A4, the bacteria were cultured for AMD production which was then recovered in an organic layer (see Fig. 2). They contained pMevT, pMBIS and pADS (see Fig. 1) and were grown in C medium containing 3.4% glycerol (a carbon source). When the cell culture had an OD_{600} of about 0.25, IPTG and varying amounts of mevalonate were added. Fig.2 shows concentrations of AMD produced by the culture cells over time.

15. In the board's view, a host cell which is cultured without addition of fresh media or nutrients (i.e. feed) cannot fall within the scope of the claims, as no feed is added. A feed cannot be reduced if it is absent. For this reason alone document A2 cannot anticipate the method of claim 1.

15.1 Even if the MSGR can be calculated based on the growth curve shown in Figure 3 of document A2, resulting in a MSGR on the cell growth curve (o) and (□) in low carbon and high carbon medium of 0.217 [s^{-1}] and 0,385 [s^{-1}] respectively, the cell culture in low carbon did not receive any feed. The specific cell growth rate during the exponential growth phase of the cell culture in low carbon was 100% (MSGR) and thus was not maintained at a level of less than 90% of the MSGR. In spite of the relative ratio of the growth rates between the carbon medium conditions of 0.2171/0.3856 = 56%, demonstrating that the low carbon source in the culture was maintained at a level less than 90% of the MSGR, no feed was added during the whole cell culture carried out in low carbon source. Under these circumstances, it is of no relevance to determine whether the production of amorphadiene (AMD) is improved or not.

15.2 As the MSGR and maintenance of a carbon source at a level providing for a specific cell growth less than 90% of the MSGR value is not deemed to be an unusual parameter, the burden of proof cannot be reversed (see decisions T 388/15 and T 1406/14 and item 11.3 above).

15.3 Documents A3 and A4 disclose batch culture methods. For this reason alone, respondent's novelty objection cannot succeed.

15.3.1 In addition, in document A4, Figure 2 provides no information with respect to the growth rate of the single culture with cells comprising all of the MEV pathway enzymes, let alone the MSGR determined under unrestricted carbon source. The growth curve shown in Figure 3 was obtained with cells not comprising a complete mevalonate pathway (i.e. pMevT, pMBIS and pADS) and in consequence cannot be mapped onto the different culture conditions of Figure 2.

15.4 Hence, in the board's view, there is no convincing evidence, going beyond reasonable doubt, that the methods disclosed in documents A2 to A4 include all the method steps of claim 1. Thus, none of the documents A2 to A4 is considered to anticipate the subject-matter of claim 1. The main request fulfils the requirements of Article 54 EPC.

Inventive step (Article 56 EPC)

16. It is common ground between the parties that document A2 represents the closest prior art for claim 1.

16.1 Document A2 relates to the production of Amorpha-4,11-diene (AMD), an isoprenoid, in *E. coli* host cells. The bacterial host cells comprised a mevalonate (MEV) pathway, wherein all of the pathway enzymes were under the control of at least one heterologous transcriptional regulator (see Table I, page 685, col. 2, second paragraph). The host cells were cultured in TB medium, or in TB medium supplemented with glycerol (TBG) once the cells entered the stationary phase (see page 688, col.1, Figure 3).

16.2 The method step (b) of claim 1, culturing the host cells in a medium comprising a reduced feed of a carbon

source, is the distinguishing feature resulting in an improved production of isoprenoids.

16.3 Based on the experimental data provided in Example 12 of the patent and the arguments submitted under Article 83 EPC above, the technical effect is considered to be achieved.

16.4 Starting from document A2, the technical problem underlying the method of claim 1 may be formulated as the provision of a method for improved isoprenoid production.

Obviousness

16.5 The question that remains to be answered is whether the skilled person starting from the teaching of document A2 and faced with the technical problem formulated above was motivated to solve the technical problem by modifying the culture conditions of the host cell as proposed by claim 1.

16.5.1 Document A2 mentions that further optimizing bioreactor media and conditions to use a medium with balanced nutrients and optimal carbon delivery was planned. "A future, detailed study of medium development should further increase product concentrations" (document A2, page 690, right column, middle of the page).

16.5.2 Even if the skilled person reading these lines may have been motivated to optimize the composition of the medium for improving the isoprenoid production yield, there is no indication whatsoever how this optimization may be achieved.

16.5.3 It is true that the skilled person knew that the volumetric yield of recombinant products could be improved by controlling the specific growth rate and the substrate concentration while the formation of inhibitory by-products could be minimized in fed-batch cultures (see document A13, abstract; document A13, page 1550, col.2, last paragraph; page 1551, col.2 last full paragraph; document A22, page 1004, col.1). In yeast production, ethanol could be produced from excess glucose even if the dissolved oxygen concentration was sufficient. This phenomenon was known as "Crabtree effect" and arises in *E. coli* as well (see document A13, page 1550, col.2 2nd full paragraph; document A16, abstract). It consists of the repression of the consumption of an energy source (respiration) by another energy source (fermentation) and was explained by the repression of the respiratory enzymes synthesis in exponentially growing yeast or bacteria at high glucose concentrations (see documents A16 and A22). A straightforward approach to prevent acetate formation was to force glucose limited cells to grow below the threshold specific growth rate. Cells could be constrained in this way merely by restricting the supply of glucose; therefore, a fed-batch process is superior to a batch process for protein production (see document A26).

16.5.4 There is no teaching in document A4 using a batch culture fermentation - not a fed-batch fermentation - that the Crabtree effect was actually a problem in the production of isoprenoids - not of proteins - and that any potentially supplied feed medium is important and must be controlled.

16.5.5 Although the Crabtree effect was known in the art (see documents A13, A15, A16, A22 and A26) to have an impact

on the production of recombinant proteins or enzymes, on the Baker's yeast's fermentative capacity (i.e. ethanol production), on *E. coli's* growth rate and biomass yield, none of these documents teaches or suggests that the Crabtree effect is or has a limiting and/or detrimental effect on the host cells defined in claim 1 for the production of isoprenoids.

16.5.6 Even if document A13 discloses that the Crabtree effect affects the production of proteins and that the host cells in claim 1 express recombinant enzymes, document A2 fails to teach or suggest that the expression of one or more of the heterologous enzymes of the mevalonate pathway was compromised and had to be increased in order to achieve an improved production of isoprenoids. There is even less of an indication as to why a suboptimal feed of carbon source would have found its way only partially to the production of proteins while isoprenoid production was improved.

16.6 Starting from the closest prior art document A2, there is equally no indication and motivation why the skilled person would turn to document A1 to provide for a method for improved production of isoprenoid. Indeed, document A1 relates to a method for cultivating yeast or bacteria in a culture medium, wherein the carbon source concentration in the culture medium is maintained at a constant low level. The compound to be produced was L-lysine and other amino acids, such as glutamic acid (see Example 1).

16.6.1 The board finds no evidence in documents A2 or A1 that the production of isoprenoids in host cells should be identical or similar to the production of glutamic acid or other amino acids. There is no evidence either why the method for producing isoprenoids using host cells

in document A2 should be improved, if the skilled person followed the teaching of documents A3 or A4, using a batch fermentation process - lacking any feed of carbon - in which the basal medium is supplemented with mevalonate, to balance the flow of carbon through the mevalonate pathway, leading to improved growth and production of isoprenoids in *E. coli* (see document A4, bridging sentence col 1 and 2 on page 194).

16.6.2 Although the skilled person knew that the balancing of nutrients was important when recombinant host cells were fermented using a fed-batch method for producing isoprenoids and that document A5 relates to fed-batch techniques in microbial processes, there is nevertheless no indication why the skilled person should turn to document A5 if the method for producing isoprenoids has to be improved. Document A5 does neither address the production of isoprenoids using recombinant host cells nor does it provide any indication that isoprenoid production could be improved by decreasing the feed of carbon source with a reasonable expectation of success.

16.6.3 Finally, there is no apparent motivation for the skilled person to combine the teaching of document A2 with that of A6, which concerns the cultivation of algae -which are neither bacteria nor fungi- and which do not comprise one or more heterologous enzymes of the mevalonate pathway under the control of at least one heterologous transcriptional regulator.

16.7 Thus, the subject-matter of claim 1 satisfies the requirements of Article 56 EPC. This applies, mutatis mutandis, also to the subject-matter of dependent claims 2 to 10.

17. The board concludes that the claims according to the present main request and the invention to which they relate, and the description adapted thereto, which had not been objected to, comply with the requirements of the EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent on the basis of
   - claims 1 to 10 of the main request submitted with appellant's letter dated 1 March 2022, and
   - a description and figures as granted but with replacement description pages 3, 4, and 20 submitted with appellant's letter dated 1 March 2022 and with replacement page 3a submitted with appellant's letter dated 3 March 2022.

The Registrar: The Chairman:

M. Schalow B. Stolz

Decision electronically authenticated