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**Datasheet for the decision
of 30 April 2021**

Case Number: T 1587/17 - 3.3.08

Application Number: 07777278.8

Publication Number: 2021486

IPC: C12P7/02, C12P7/04, C12P5/02,
C12P5/00, C12M1/02, C10L1/02

Language of the proceedings: EN

Title of invention:
Bio-organic compound production system

Patent Proprietor:
Amyris, Inc.

Opponent:
Ajinomoto Co., Inc.

Headword:
Production system/AMYRIS

Relevant legal provisions:
EPC Art. 54, 56, 83, 108, 114(2), 123(2)
EPC R. 126(2), 131(2), 131(4), 134(1)
RPBA Art. 12(4)
RPBA 2020 Art. 25(2)

Keyword:

Appeal - admissible - (yes)

Admission of a new line of argument - (no)

Main request - requirements of the EPC met - (yes)

Decisions cited:

T 0128/87, J 0019/90, T 0371/92, T 1200/01, T 2454/11

Catchword:



Beschwerdekammern
Boards of Appeal
Chambres de recours

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Case Number: T 1587/17 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 30 April 2021

Appellant: Ajinomoto Co., Inc.
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Decision under appeal: **Interlocutory decision of the Opposition
Division of the European Patent Office posted on
7 April 2017 concerning maintenance of the
European Patent No. 2021486 in amended form.**

Composition of the Board:

Chairman B. Stolz
Members: M. Montrone
L. Bühler

Summary of Facts and Submissions

- I. The appeal lies against the decision of an opposition division to maintain the European patent No. 2 021 486 in amended form. The patent was filed under the PCT and published as international patent application WO 2007/139924 (hereinafter the "patent application").
- II. The opposition division considered the main request, and auxiliary requests 1 and 2 to contravene Article 123(2) EPC, while auxiliary request 3 was held to fulfil the requirements of the EPC.
- III. The opponent (hereinafter the "appellant") paid the appeal fee on 19 June 2017 by way of debit order. This order was sent as part of a grouped filing for 19 files accompanied by a list No. 486/2017 which specified the documents enclosed therein and the European patent applications and patents to which these documents related. Item 17 in this list concerned the opposed patent. The debit order for the appeal fee (abbreviated "Abb." = "Abbuchung" / "debit") was mentioned in the column "Zahlung" ("payment"). A further document referred to as "appeal" ("Beschwerde") concerning the opposed patent was included in this list under item No. 17. While a scanned copy of the debit order was entered in the European Patent Register with date of 19 June 2017, a document corresponding to the "appeal" mentioned in the list of enclosures of the grouped filing of 19 June 2017 was missing in the electronic file.
- IV. Following an inquiry by a formalities officer on 26 June 2017 about the payment of an appeal fee without a corresponding notice of appeal, the appellant's

representative replied on 27 June 2017 that the notice of appeal had been filed as part of a grouped filing No. 486/2017 on 19 June 2017. The receipt of this filing had been acknowledged by the EPO by way of a date stamp on the list of enclosures for this grouped filing. An investigation within the EPO was carried out in order to find the allegedly transmitted notice of appeal. However, no document corresponding to said notice could be found.

- V. By letter dated 14 August 2017, the appellant filed a copy of the notice of appeal as allegedly filed on 19 June 2017 as well as a copy of the list of enclosures of the grouped filing No. 486/2017 stamped by the EPO Munich. It provided arguments as to the receipt of the notice by the EPO.
- VI. With their statement of grounds of appeal, the appellant submitted arguments under added subject-matter, sufficiency of disclosure, novelty and inventive step against the subject-matter of auxiliary request 3 as maintained by the opposition division.
- VII. In a communication, the board, based on the facts on file, assessed the issue of whether or not the appellant's appeal had been duly filed within the time-limit set in Article 108 EPC. The board arrived at the preliminary opinion that the appeal was admissible and invited the parties to submit comments.
- VIII. The appellant agreed with the board's preliminary finding on this issue. The patent proprietor (hereinafter the "respondent") submitted that the evidence provided by the appellant for filing the notice together with the grouped filing No. 486/2017

within the time-limit of Article 108 EPC was merely circumstantial, and hence, not convincing.

- IX. In reply to the appellant's statement of grounds of appeal, the respondent submitted a main request (corresponding to auxiliary request 3 as maintained by the opposition division), and an auxiliary request. Further, the respondent provided counter-arguments to the issues raised by the appellant.
- X. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's provisional, non-binding opinion.
- XI. In reply, the respondent submitted a further auxiliary request.
- XII. Oral proceedings before the board were held on 30 April 2021 by video conference as requested by the parties.
- XIII. Claim 1 of the main request reads:

"1. A bio-organic compound production system comprising:
a. a vessel having a capacity of at least 100 liters;
b. an aqueous medium, within the vessel, forming a first phase;
c. a plurality of genetically modified bacterial or fungal host cells, within the aqueous medium, capable of converting a carbohydrate carbon source present in the aqueous medium into at least one bio-organic compound having at least ten carbon atoms; and

d. a liquid organic second phase, comprising the at least one bio-organic compound in an amount of at least 90%, in contact with the first phase; wherein the at least one bio-organic compound is an isoprenoid compound; and wherein the organic second phase is spontaneously formed".

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

- D1: Newman J.D., *et al.*, *Biotechnology and Bioengineering*, 2006, Vol. 95(4), 684-691;
- D2: Tecelao C.S.R., *et al.*, *Journal of Molecular Catalysis B: Enzymatic*, 2001, Vol. 11, 719-724;
- D3: US5763237 (published 9 June 1998);
- D5: Stanbury P.F., *et al.*, *Principles of Fermentation Technology*, 2nd edition, 1995, 167-170, 269-271;
- D6: Leon, R. *et al.*, *Biomolecular Engineering*, 2003, Vol. 20(4-6), 177-182;
- D8: Amyris, Inc., *Integrated Biorefinery Project Summary*, 30 June 2013;
- D9: Exhibit A;
- D10: Exhibit B;
- D11: Joshua Leng's declaration, dated 16 March 2016;
- D12: Christi Y: "Fermentation (industrial): Basic considerations", Robinson R., Batt C., Patel P. (Editors): "Encyclopedia of Food Microbiology",

1999, Academic Press, London, 663-674.

- XV. The appellant's submissions, insofar as relevant to the present decision, may be summarised as follows:

Admissibility of the appeal

The notice of appeal was duly filed with the EPO on 19 June 2017 and thus within the time-limit of Article 108 EPC. The notice was sent together with the payment sheet for the payment of the appeal fee as part of a grouped filing No. 486/2017. The stamp "EPO - Munich 10x 19. Juni 2017" on the list of enclosures accompanying the grouped filing was proof of receipt by the EPO on the same day of all enclosures specified in said list. Further proof for receipt of the notice was the fact that the payment sheet for the appeal fee that had been sent together with the notice was received, stamped, scanned and entered in the register by the EPO. The signature next to item No. 17 of the list of enclosures furthermore showed that the respective enclosures had been carefully checked by the appellant's representative before dispatch. The whole circumstantial evidence was consistent with and further confirmed by the acknowledgement of receipt of the notice of appeal by way of stamping and returning the list of enclosures.

Admission into the proceedings of a new line of argument under added subject-matter

A specific objection under added subject-matter was not raised against the feature "at least ten carbon atoms" in claim 1 during the first instance proceedings, but submitted for the first time in the statement of grounds of appeal only. However, this feature in claim

1 was generally attacked in the previous proceedings in the context of the various other objections submitted under added subject-matter. Therefore this line of argument was not new but merely focused on aspects of earlier objections.

Main request

Added subject-matter

Several objections under added subject-matter were raised.

Firstly, the feature "*a liquid organic second phase, comprising the at least one bio-organic compound in an amount of at least 90%*", combined with the indication that the compound "*is an isoprenoid*" in step d. of claim 1 did not have a basis in the patent application. Although the subject-matter of claims 7 and 15 as filed mentioned an "*isoprenoid*", and an organic phase that comprised a concentration of "*at least 90%*" of a bio-organic compound, the subject-matter of these two claims as filed could not be combined with each other. This was so because claims 7 and 15 as filed referred to claim 1 as filed, while claim 15 as filed lacked a back-reference to claim 7 as filed.

Secondly, the feature "*genetically modified bacterial or fungal host cells*" in step c. of claim 1 was derived from selections of different passages of the patent application. Although paragraphs [0083] to [0088] of the patent application mentioned various host cells, they provided no pointers to the specific modified host cells cited in claim 1, thereby creating a novel subgroup of host cells. Moreover, these paragraphs were silent on the production of isoprenoids in a

concentration of "at least 90%". The combination of specific host cells that produced isoprenoids in a defined minimal amount was also not derivable from paragraphs [0051], [0055], or [0092] of the patent application. While paragraph [0092] of the patent application mentioned isoprenoids, it was silent on a concentration of this compound. Paragraph [0055] of the patent application disclosed various concentration ranges of a bio-organic compound in an organic phase, but was silent on isoprenoids, including the use of a preferred concentration range.

Thirdly, the feature "*the organic second phase is spontaneously formed*" in claim 1 lacked a basis in the patent application. While paragraph [0058] of the patent application mentioned a spontaneously formed organic phase, this type of formation was one of several disclosed alternative mechanisms. A pointer for its preferred use was, however, lacking in this paragraph. Nor was a pointer derivable from Example 23 of the patent application, which disclosed merely a hypothetical working example that was silent on an amount of "at least 90%" isoprenoids. Furthermore, this Example described two alternative organic phase separation mechanisms without pointing as being preferred to the use of settlement and decantation. Moreover, since decantation required an active intervention, a phase separation based thereon was not spontaneous. Lastly, paragraph [0003] of the patent application provided no pointer for a spontaneously formed organic phase too. Neither disclosed this paragraph a spontaneously formed organic phase, nor was it an inherent feature of industrial-scale systems because it was not the necessary result thereof.

In summary, the combination of these three features in claim 1 created a novel subgroup which added subject-matter to the patent application.

Sufficiency of disclosure

The term "*spontaneously formed*" in claim 1 implied that the separation between the organic and aqueous phases did not require any external activity or intervention. The patent application was silent on a teaching how to perform a spontaneous phase separation, in particular in an industrial scale, as implied by a minimum vessel size of 100 l cited in claim 1. The working examples in the patent application used small cultivation vessels only. Information for scaling these processes up was not provided. Example 23 of the patent application mentioned settlement and decanting of the organic phase, if the titer of the compound was large enough to saturate the medium to form a second phase (see paragraph [0321]). However, this Example neither disclosed how an organic phase could be decanted from a 100 l fermenter, nor which titer (i.e. concentration) formed spontaneously an organic phase. The patent application was also silent on host cells suitable for the claimed purpose. In these circumstances the skilled person had to start its own research project to find suitable conditions for a spontaneous phase separation which amounted to undue burden. In such a situation where the patent application lacked any teaching for the skilled person how a claimed invention could be carried out, the burden of proof was not on the opponent-appellant to submit verifiable facts in support of insufficiency.

The respondent's submission of supplementary experimental evidence (see Exhibits A and B as

documents D9 and D10, respectively) did not discharge their burden of proof. The experiments disclosed in these documents used vessel sizes that were far smaller than the 100 l cited in claim 1. Moreover, the data was obtained from a single transformed host cell (yeast) producing a single isoprenoid (farnesene). A single example, however, was not sufficient to support the enablement of the system for the whole breadth of claim 1. Furthermore, documents D9 and D10 were silent on technical details of the experiments, for example, the sampling, the sample composition, and the cultivation conditions. Moreover, since the samples were removed from the fermenter and transferred into a different vessel for settlement, this step might have induced the phase separation. Therefore, since the experimental set-up in documents D9 and D10 differed from the claimed system, the finding of a spontaneous phase separation in these documents was no evidence that the same happened in the claimed system too. Furthermore document D11 concerned an expert declaration that commented on the data in documents D9 and D10. The expert stated that the phase separation into layers required a settlement of 24 hours, which was too long for a spontaneous formation. Lastly, also document D8 provided no evidence that a spontaneous phase separation occurred in the claimed system, since it applied a centrifugation step for this purpose (see page 37), i.e. the phase separation was induced and not spontaneous.

Novelty

The subject-matter of claim 1 lacked novelty over documents D1 to D3. Although in the decision under appeal the opposition division held that *inter alia* the vessel size of 100 l in claim 1 distinguished the

claimed system from documents D1 to D3, this feature was a mere arbitrary design option which the skilled person would have read into these documents.

Inventive step

Documents D1 to D3, or D6 represented the closest prior art. The claimed subject-matter was obvious for the skilled person starting from documents D1 to D3 in combination with documents D5 or D12, or starting from document D6 in combination with either of documents D1 to D3.

Document D1 disclosed a two-phase aqueous-organic system for the production of amorpho-4,11-diene (a volatile isoprenoid compound) using a genetically modified *Escherichia coli* (*E. coli*) host cell. The document mentioned "*large-scale fermentations*" (see abstract). Document D1 further disclosed a "*spontaneously formed*" organic second layer as cited in claim 1, because this term encompassed any phase separation mechanism, which included the formation of a condensate of a volatile isoprenoid in a cold trap.

Therefore, the claimed system differed from document D1 only in the use of a vessel size of at least 100 l. Unexpected technical effects were not ascribable to this distinguishing feature. The technical problem was hence the provision of a scaled-up isoprenoid production system.

Since vessel sizes for industrial-scale fermentations were known from the prior art (see document D5, pages 168 and 169, Figures 7.1 and 7.2; document D12, page 664, column 2, fourth paragraph), and even pointed at in document D1 (see abstract), the skilled person would

have arrived in an obvious manner at the claimed system. Even if the condensate of document D1 was not encompassed by the claimed system, the system of claim 1 encompassed a settling and decanting step for forming an organic phase of isoprenoids (see Example 23 of the patent application) which had no advantageous or unexpected properties compared to the use of the simple cold trap system of document D1. The same arguments applied, if documents D2 or D3 were selected as the closest prior art.

Document D6 disclosed a two-phase production system for β -carotene (likewise an isoprenoid). Microalgae were grown in an aqueous medium which intracellularly accumulated β -carotene. An overlay of a liquid organic phase in contact with the aqueous phase was used for extracting the β -carotene from the cells in an amount of at least 90%. The second organic phase was spontaneously formed. Although a vessel with a volume of at least 100 l was not disclosed in document D6, vessels of that size for large-scale fermentations were known to the skilled person (see documents D5, or D12). The claimed system differed from document D6 only in the use of a bacterial or fungal host cell instead of a microalgae for producing isoprenoids.

The technical problem to be solved was hence the provision of an alternative system for the production of an isoprenoid compound.

Document D1, for example, was also directed to a two-phase system for the microbial production of an isoprenoid using an engineered *E. coli* strain. Therefore, the use of such a host cell in the claimed system was an obvious alternative for the skilled person starting from document D6.

Similarly, document D2 related to the microbial conversion of carveol to carvone (another isoprenoid compound) in a two-phase production system using the bacterium *Rhodococcus erythropolis*, while document D3 described the microbial conversion of limonene (likewise an isoprenoid) in a two-phase production system using an engineered *E. coli* strain.

For the reasons set out above for document D1, the selection of alternative host cells to produce isoprenoids was an obvious modification for the skilled person to arrive at the claimed system. The subject-matter of claim 1 therefore lacked an inventive step in light of the teaching of document D6 combined with documents D2 or D3 too.

XVI. The respondent's submissions, insofar as relevant to the present decision, may be summarised as follows:

Admissibility of the appeal

The burden of proof was on the appellant who had to prove to the satisfaction of the board that the EPO had received the notice of appeal in due time (T 2425/11). Proof of a higher probability that an item was filed than that it was not filed, was not sufficient. The fact that the cover sheet of the grouped filing No. 486/2017 was stamped and returned, said nothing about what the enclosures in relation to any individual case were. The stamped cover sheet only showed that the bundle contained "something" in relation to each listed application. This included the fee payment sheet, which was also stamped, scanned, and appeared on the register. However, there was no proof that a notice of appeal was enclosed. The relevant entry on the enclosure list referred to "Beschwerde", meaning

"appeal", and "ABB", meaning debit, only demonstrated that something relating to an appeal was filed and that a fee was being paid, which is consistent with only a fee payment sheet for the appeal fee being filed. There was no reference to a "Beschwerdeschrift" (notice of appeal) being filed. In sum, confirmation of the entire contents listed on the cover sheet of the grouped filing No. 486/2017 did not constitute proof of the fact that the notice of appeal was comprised in those contents. The only evidence provided (same date stamp on the cover sheet and on the payment sheet, staple holes on the payment sheet, signature next to item 17 of the cover sheet) was circumstantial and did not meet the high standard of proof required.

Admission into the proceedings of a new line of argument under added subject-matter

The new line of argument under added subject-matter against the feature "*at least ten carbon atoms*" of claim 1 was not raised by the appellant during the first instance proceedings. Claim 1 of the present main request differed from claim 1 as granted only in that the feature "*40-99%*" has been deleted. Therefore the contested feature was present in claim 1 since the onset of the opposition proceedings, and reasons for not raising an objection against it were not apparent. The new line of argument against the "*at least ten carbon atoms*" feature in claim 1 was also not implicitly raised in view of the appellant's first instance submissions on this issue. This feature was not technically linked to any of the other contested features in claim 1, in particular not to a liquid organic phase comprising isoprenoids in an amount of at least 90%. It was independent therefrom. Since the appellant should have raised this objection already

during the first instance proceedings, there was no justification for submitting it for the first time with their statement of grounds of appeal.

Main request

Added subject-matter

Firstly, the feature "*a liquid organic second phase, comprising the at least one bio-organic compound in an amount of at least 90%*", wherein the compound "*is an isoprenoid*" in step d. of claim 1 was disclosed in claims 7 and 15 of the patent application. The reference to an "*isoprenoid compound*" in a concentration of "*at least 90%*" in claims 7 and 15 as filed, respectively, provided a pointer to the skilled person that both features were preferred. Isoprenoids were used interchangeably with bio-organic compounds, and, moreover, were disclosed as the sole exemplified bio-organic compound in the patent application (see paragraphs [0051], [0054], and [0092]). Since the patent application indicated this feature combination as preferred, their combination resulted not from the selection of different unrelated description parts.

Secondly, the combination of "*genetically modified bacterial or fungal host cells*" in step c. of claim 1 was based on paragraph [0021] of the patent application which mentioned that the terms "*host cell*" and "*microorganism*" were used interchangeably, and on paragraph [0083] of the patent application which disclosed that a "*host cell is a genetically modified host microorganism*". These modified cells were the only exemplified cells in the patent application. Bacteria and fungi as host cells were mentioned in paragraphs [0084] to [0091] of the patent application. Moreover,

the working examples of the patent application disclosed exclusively either genetically modified bacteria or fungi as host cells for producing isoprenoids. Since the patent application provided clear pointers for using the host cells referred to in claim 1, their combination in a system for producing isoprenoids in a concentration of at least 90% was not an arbitrary selection of features derived from different unrelated patent application parts.

Thirdly, the feature "*spontaneously formed*" in claim 1 was derivable from paragraph [0058] of the patent application which disclosed that phase separation of the organic phase from the aqueous phase occurred by two mechanisms only, either spontaneously or induced. Since two alternative separation mechanisms were disclosed only, both were preferred. Moreover, a selection of one alternative out of two was not a selection from a list. Example 23 of the patent application corroborated the preferred use of a spontaneous phase separation, since it disclosed as one of two alternatives, a settlement and decanting of an organic isoprenoid phase.

Sufficiency of disclosure

The appellant's arguments were not supported by verifiable facts. Example 23 of the patent application disclosed in paragraph [00321] the production of various isoprenoid compounds having at least 10 carbon atoms. This involved *inter alia* the spontaneous formation of an organic second phase by settling and decanting, if the isoprenoid titer was high enough to saturate the medium. Accordingly, the patent application mentioned experimental conditions allowing the spontaneous formation of a second organic phase.

Further, the patent application disclosed various mechanisms for forming spontaneously an organic phase (see paragraph [0058]). Thus, based on the disclosure in the patent application, the spontaneous formation of an organic isoprenoid phase was at least plausible for the claimed system.

In these circumstances the case law allowed the submission of supplementary experimental evidence to confirm a claimed technical effect.

The supplementary evidence in documents D9 and D10 disclosed that the production of the isoprenoid farnesene by a genetically modified yeast cell resulted in the spontaneous formation of an organic layer after a gravity settlement of 24 hours. This phase typically comprised more than 90% farnesene (see document D8, page 38, Table A.7). Although the sampling was not described in detail in documents D9 and D10, the samples used for the phase separation were identical to the fermenter broth, i.e. the sample was representative of the broth. There was no reason apparent why the organic phase separation in documents D9 and D10 should not likewise occur in a fermenter vessel of at least 100 l as cited in claim 1. Document D8 disclosed a pilot plant with 300 l fermenters for the microbial production of farnesene. It confirmed that the skilled person did not encounter any technical problems in reducing the claimed invention into practise. The centrifugation step disclosed on page 37 clarified the syrup prior to the fermentation. This step was not used for the phase separation as alleged by the appellant.

The appellant did not submit a single example of a non-working embodiment falling within the scope of claim 1. The finding in documents D9 and D10 that a 24 hour gravity settlement was needed to form a layer of the organic phase was irrelevant for sufficiency, since a

spontaneous formation was not time restricted. Moreover, claim 1 was not directed to the spontaneous formation of a layer, but of an organic phase that was in contact with the aqueous phase. A separation did not require that two layers were formed.

Novelty

The production system of claim 1 was novel over documents D1 to D3, at least because it used a vessel with a minimum size of 100 l. Contrary to the appellant's view, the vessel size was not an arbitrary design option, but a structural feature of claim 1.

Inventive step

Document D1 represented the closest prior art, not document D6. The production system of claim 1 differed from the system in document D1 in at least three features, while it differed from document D6 in at least five features.

The distinguishing features between the claimed system and document D1 were:

- (i) a larger vessel size (100 l versus 10 l, see document D1, page 685);
- (ii) the spontaneous formation of the second organic phase instead of either adding an overlay of an organic phase consisting of dodecane (see document D1, page 685, column 2, last paragraph), or using a cold trap cooled with dry ice and ethanol (see document D1, page 686, column 1, second paragraph);
- (iii) a liquid organic phase of at least 90% isoprenoid instead of using a liquid organic phase of dodecane comprising a low concentration of an isoprenoid, or if

a cold trap was used, a liquid organic phase that was not in contact with the aqueous phase.

These differences resulted in the provision of a simplified isoprenoid production system. Due to the spontaneous formation of the organic phase the isolation of isoprenoids required neither the addition of organic solvents nor technical means, such as the cold trap. The need for less process steps increased the efficiency of the production system at lower costs.

The technical problem was therefore the provision of a simplified isoprenoid production system.

The production system of claim 1 solved this problem as shown in Example 23 of the patent application.

Documents D8 to D11 provided further evidence that this problem was solved by the claimed system.

The production system of claim 1 as a solution to this problem was also not obvious for the skilled person starting from the system of document D1, since none of the available prior art documents provided a pointer that isoprenoids in an amount of at least 90% formed spontaneously an organic phase in an aqueous medium.

- XVII. The appellant requested that the decision under appeal be set aside and that the patent be revoked.
- XVIII. The respondent requested that the appeal be rejected as inadmissible. In the alternative, they requested that the appeal be dismissed. They further requested not to admit the late filed objection under Article 123(2) EPC in respect of the feature "*having at least 10 carbon atoms*" cited in claim 1.

Reasons for the Decision

Admissibility of the appeal

1. The time limit pursuant to Article 108, first sentence, EPC for filing an appeal against the interlocutory decision of the opposition division posted on 7 April 2017 concerning the maintenance of European patent No. 2 021 486 in amended form expired on 19 June 2017 (Rule 131(2), second sentence, and (4) EPC, Rule 134(1) EPC, and Rule 126(2) EPC). The only entry in the European patent register on 19 June 2017 for the opposed European patent No. 2 021 486 is a debit order for the appeal fee by the appellant-opponent's representative (EPO form 1010, "Payment of fees and expenses"). Payment of an appeal fee does not in itself constitute filing of an appeal (established case law following J 19/90 and T 371/92, OJ 1995, 324). Therefore, an appeal against the decision of the opposition division dated 7 April 2017 is deemed to have been filed only if a notice of appeal was received by the EPO on or before 19 June 2017.

2. The **legal burden of proof** is on the party who derives rights from an alleged positive fact. Accordingly, the party asserting a right must prove the facts giving rise to that right, while the burden of proving the facts nullifying a right lies with the party disputing its existence. Therefore, the legal burden of proof for the fact that a filing complying with a time-limit has been received by the EPO, rests on the sender. In the present case, the burden thus lies on the appellant to establish that their notice of appeal against the decision of the opposition division posted on 7 April 2017 concerning the maintenance of European

patent No. 2 021 486 in amended form reached the EPO within the time limit pursuant to Article 108, first sentence, EPC (T 1200/01, Reasons, point 4; T 2454/11, Reasons, point 2.1).

3. With respect to the question of **standard of proof**, i.e. the degree of certainty that is required for the board to conclude that an alleged fact is made out, the respondent stressed that decision T 2454/11 establishes that the sender has to prove receipt of a document allegedly sent to the EPO "to the board's satisfaction" which implied a high standard. However, decision T 2454/11 does not explain what degree of certainty is required by the standard "satisfaction of the board" (see Reasons, points 2.5 and 2.6). It is therefore necessary to determine the required standard.

- 3.1 Decision T 2454/11 cannot be understood to require absolute certainty. Absence of slightest doubts would indeed be unreasonable since it would be impossible to meet such a stringent standard of proof. The board in case T 2454/11 nevertheless intended to distinguish the standard of proof applied by it from that applied in case T 1200/01. It appears that the board in case T 2454/11 took issue with a standard of "high probability" in the sense of a fact being "more likely than not". However, as becomes evident from the Reasons, point 4 of decision T 1200/01 (referring to "proof of higher probability") and the analysis of the assessment of the evidence by the board in that case (Reasons, points 6 to 8), the board in case T 1200/01 applied a more stringent standard of proof, namely that of "preponderant probability". Evaluating and weighing the conflicting evidence, the board assessed whether there remained reasonable doubts that the filing had occurred as alleged by the appellant.

- 3.2 The standard of "preponderant probability" requires a high degree of probability (see e.g. T 128/87, OJ EPO 1989, 406, point 6.1). The possibility that the alleged fact might not have materialised should only be remote or marginal. In other words, a fact is proven if the court no longer has serious doubts about the existence of the alleged fact, or if any remaining doubts appear to be slight. In the board's opinion, this equates to the standard requiring absence of "reasonable doubts" in which case the alleged fact is made out to the "satisfaction of the board" as in case T 2454/11.
4. In the present case, the appellant has proven "to the satisfaction of the board" that a notice of appeal regarding the decision of the opposition division posted on 7 April 2017 concerning the maintenance of European patent No. 2 021 486 in amended form reached the EPO on 19 June 2017.
- 4.1 On 19 June 2017, the opponent paid the appeal fee by way of debit order on EPO form 1010. This fee payment sheet refers to the opposed patent by both its application number (No. 07777278.8) and publication number (No. 2 021 486). The sheet is signed and dated by the appellant-opponent's representative and date stamped "19 June 2017" by the EPO. A scanned copy is part of the electronic file. The fee payment sheet was part of a grouped filing of the representative's office on 19 June 2017. The filing was accompanied by a list numbered 486/2017 specifying the documents enclosed in the grouped filing and the European patent applications and patents to which these documents relate. Item No. 17 in this list indicates the application number 07777278.8 of the opposed European patent. This entry specifies that the corresponding enclosure in the

grouped filing is an "appeal" ("Beschwerde") and that payment is made by debit order ("ABB" which is the abbreviation for "Abbuchung"). The latter document undoubtedly reached the EPO while the document referred to as "appeal" is missing in the electronic file. However, by date stamping and returning the list of enclosures of the grouped filing No. 486/2017 the EPO acknowledged receipt of the complete enclosures. This holds even more true considering that it was explicitly requested on the list of enclosures to return the list immediately. Had an item been missing, the EPO should not have returned the stamped list without any proviso. As is evidenced by the date stamps on individual items of the grouped filing No. 486/2017, like the payment sheet for the appeal fee in the present case, the individual enclosures were sorted and processed by the EPO's mail room. Any missing item would have immediately become apparent. The list of the grouped filing bearing the date stamp of the EPO is confirmation and proof that the items on that list reached the EPO on 19 June 2017. Indeed, such a confirmation of receipt by the EPO was considered essential but missing in the case underlying decision T 2454/11 (see Reasons, point 2.3, first paragraph, and point 2.7, third paragraph: "Furthermore, neither the three documents nor D1 contain any indication to the mail room to acknowledge their receipt. Moreover, the appellant itself admits that document D1, which lists the documents contained in the UPS envelope, was neither stamped by the EPO nor returned to the office of its representative.", translation by the board).

4.2 The respondent argued that the reference to "Beschwerde" ("appeal") under item 17 of the list of enclosures was no proof that a notice of appeal had in fact been enclosed. However, the board has no serious

doubts that the notice of appeal, a copy of which was filed on 14 August 2017, was part of the filing received by the EPO. The entry "Beschwerde" in the column "attached documents" refers to a document different from the fee payment sheet which is on file. When referring to a document, the term "Beschwerde" in German either means "Beschwerdeschrift" ("notice of appeal") alone or includes additionally the statement of grounds of appeal. It cannot be understood to refer to the payment sheet, let alone to any other document in this context. Therefore, there are no reasonable doubts that the payment sheet on file was sent together with a notice of appeal as part of item No. 17 of the grouped filing of 19 June 2017. The short signature of a secretary next to item No. 17 of said list shows that the respective enclosures had been checked at the representative's office. The possibility that the notice of appeal was not included in the grouped filing is therefore very remote. In any case, the question whether the envelope that left the representative's office contained all items listed in the list of enclosures of the grouped filing No. 486/2017 is not decisive. Since the EPO did not note on the returned list of enclosures that the document listed under item No. 17 as "Beschwerde" was missing, it must have reached the EPO. The board therefore disagrees with the respondent's argument that the evidence on file is merely circumstantial. As regards the further evidence in addition to the EPO's acknowledgement of receipt, the board agrees that it is circumstantial, but notes that it is not conflicting with receipt by the EPO of a notice of appeal but rather confirming this fact.

- 4.3 In sum, the board concludes that the appellant has discharged its burden of proof. The respondent has not succeeded in raising doubts that are serious enough to

counter the evidence provided by the EPO's acknowledgement of receipt. The appeal has therefore to be considered as filed within the time-limit pursuant to Article 108, first sentence, EPC.

Admission into the proceedings of a new line of argument under added subject-matter

5. According to the established case law, the function of an appeal is to review in a judicial manner the decision under appeal taken by an examining or opposition division. The admission of *inter alia* new lines of argument into the appeal proceedings is at the board's discretion (see Articles 114(2) EPC and 25(2) RPBA 2020 in conjunction with Article 12(4) RPBA 2007).
6. It is uncontested that the appellant submitted in their statement of grounds of appeal for the first time in the proceedings a line of argument under added subject-matter against the feature "*into at least one bio-organic compound having at least ten carbon atoms*" in step c. of claim 1.
7. In essence the appellant submitted that this new line of argument was admissible because it focused on a specific aspect of a more generic objection under added subject-matter raised during the first instance proceedings.
8. The board does not agree. The contested feature in step c. of claim 1 set out above is already present in claim 1 as granted. Indeed present claim 1 differs from claim 1 as granted in the deletion of the range "40-99%" in step d. of claim 1 only, all other features, including the contested one, are identical in both claims.

- 8.1 In the board's opinion, focusing on a specific aspect of a more generic objection previously raised requires at least that the contested features are linked, for example, structurally or functionally. In the first instance proceedings the appellant raised three objections of added subject-matter against the combination of three specific features in claim 1 (see point VII, pages 7 to 9 of the notice of opposition; page 1, last paragraph of the minutes). These three lines of argument have been maintained by the appellant in the present appeal proceedings (see below).
- 8.2 The new objection of added subject-matter concerns a feature in step c. of claim 1 which relates to a functional property of genetically modified host cells, namely their ability to convert a specific carbon source into a bio-organic compound (i.e. an isoprenoid) of a defined minimal length ("*at least ten carbon atoms*"). This feature is structurally and functionally independent of the three other features in claim 1 against which the previous objections have been raised, i.e. the specific combination of genetically modified host cells, a spontaneously formed organic phase, which moreover, comprises an amount of isoprenoids of at least 90%.
9. Since the line of argument raised against the contested feature in step c. of claim 1 identified above is new, although the feature is present in claim 1 from the onset of the opposition proceedings, the appellant should have submitted an objection against it already in the first instance proceedings. Reasons for submitting it with the statement of grounds of appeal only have not been brought forward by the appellant and are not apparent to the board.

10. Accordingly, exercising its discretion under Article 12(4) RPBA 2007, the board decided not to admit this new line of argument under added subject-matter into the proceedings.

Main request

Claim interpretation - claim 1

11. Claim 1 relates to a product which is directed to a production system for bio-organic isoprenoids having at least 10 carbon atoms in length. The system is characterised by the following structural and functional features:

(i) a vessel of a defined minimum size ("*at least 100 liters*") (hereinafter the "fermenter size" feature);

(ii) a first phase of an aqueous medium in the vessel (hereinafter the "aqueous phase" feature) that contains:

(a) genetically modified bacteria or (b) fungi as host cells,

(c) which are further functionally defined as being "*capable of converting a carbohydrate carbon source present in the aqueous medium into at least one bio-organic compound having at least ten carbon atoms*";

(iii) a second phase of a liquid organic (hereinafter the "organic phase" feature) characterised by:

(a) its contact with the first phase (i.e. the "aqueous phase"),

(b) its spontaneous formation, and

(c) its isoprenoid concentration "*in an amount of at least 90%*".

12. Claim 1 encompasses as a product any production system for bio-organic isoprenoids having at least 10 carbon atoms in length with the properties defined in steps a. to d., irrespective of how the production system is provided. In other words, although claim 1 comprises a process feature for generating the organic phase ("*spontaneously formed*"), the claimed production system is limited by that process only in so far as it results in properties of the system that can be distinguished from other systems, irrespective of the way in which they have been provided.

13. Contrary to the appellant's view, the fermenter size feature in claim 1 is not an arbitrary design option, but a structural feature that limits the claimed system to those with a minimum vessel size of at least 100 l.

14. The aqueous phase feature in claim 1 is specified as an aqueous medium at a particular location (within the vessel) that contains a plurality (i.e. at least two of a single species, or a mixture of at least two different species) of "*genetically modified*" bacteria and fungi. Accordingly, natural isolates or wild-type host cells that are not genetically modified do not fall within the scope of claim 1. These cells are further functionally limited in that they have to be suitable for a particular purpose, i.e. the conversion of a "*carbohydrate carbon source*" into an isoprenoid compound of a certain minimum length ("*at least 10 carbon atoms*"). Since the term "*carbohydrate*" is normally construed by the skilled person to relate to a saccharide or sugar, bacteria or fungi that convert carbon sources other than carbohydrates into isoprenoids do not fall within claim 1.

15. The organic phase feature in claim 1 is defined by various structural and functional features.
- 15.1 Firstly, the phase is liquid, i.e. a volatile phase is not comprised by the claim.
- 15.2 Secondly, the organic phase in claim 1 is characterised in that it is "*in contact*" with the aqueous phase. This requires that both phases are in direct physical contact with each other. Other restrictions are not imposed by that term, for example, a particular order, position, or form of the two phases.
- 15.3 Thirdly, the organic phase is "*spontaneously formed*". In the board's view, the skilled person construes a spontaneous formation to take place without active external intervention, for example, by technical means (e.g. centrifugation), or chemical means (e.g. the addition of organic solvents, de-emulsifiers or nucleating agents, see paragraph [0058] of the patent application). In other words, this functional feature characterises an intrinsic property of isoprenoids of at least 10 carbon atoms in length at a concentration of at least 90% in an aqueous medium. The appellant submitted that 24 hours for forming an organic layer by gravity settlement as disclosed in documents D9 to D11 was too long to qualify as spontaneous. The board does not agree, since "*spontaneously*" does not equate to instantly and, hence, imposes no time restriction. Accordingly, organic phases according to claim 1 form immediately, or over some time.
- 15.4 It was common ground between the parties that "*spontaneously formed*" in claim 1 implied a separation of the organic phase from the aqueous phase. This separation relates to any form, including layers (see

documents D9 and D10). Since a phase separation is implied in this feature, the board does not agree with the respondent that an emulsion of an aqueous and an organic phase is encompassed by claim 1. Although in an emulsion both phases are mixed, i.e. in physical contact, they are not separated from each other.

15.5 The organic phase of step d. in claim 1 is further characterised in that it comprises "*the at least one bio-organic compound in an amount of at least 90%*". The appellant submitted that it is not clear to what the "*at least 90%*" refers, i.e. the compound or the organic phase. Therefore claim 1 encompassed an organic phase that contained (i) at least 90% of the total amount of at least one isoprenoid, leaving 10% or less in the aqueous phase, and (ii) an organic phase of at least 90% of this compound, i.e. a phase formed of at least 90% of at least one isoprenoid, and 10% or less of other organic materials only. In the board's view, the skilled person would normally construe the "*at least 90%*" in this feature to refer to the organic phase. This construction is in line with the description (see paragraph [0045] of the patent), and makes technically sense, since isoprenoids with a length of at least 10 carbon atoms are normally hydrophobe, and hence, form "*spontaneously*" an organic phase in an aqueous medium (see above).

16. In a nutshell, claim 1 is directed to a system for the production of isoprenoids of a minimum length of 10 carbon atoms by biotechnological means, in particular by using genetically modified microorganisms in an aqueous culture, which at an undefined point in time during the fermentation of a carbohydrate as the carbon source spontaneously form an organic phase of at least 90% isoprenoids. In other words, claim 1 provides a

snapshot of the production system in operation since it is defined by ingredients that are not all present in the vessel at the beginning of the fermentation but only at a later stage.

Added subject-matter

17. It is established case law that any amendment, for example, in a claim can only be made within the limits of what a skilled person would derive directly and unambiguously, using common general knowledge, and seen objectively and relative to the date of filing, from the whole of the patent application as filed.
18. The appellant submitted three objections under added subject-matter against various features in claim 1.
19. Firstly, the appellant argued that the combination of "*a liquid organic second phase, comprising the at least one bio-organic compound in an amount of at least 90%*", wherein the compound "*is an isoprenoid*" in step d. of claim 1 added subject-matter. While claims 7 and 15 as filed disclosed these features in individualised form, both claims referred back to claim 1 as filed only. Thus, claims 7 and 15 as filed provided no basis for the combined use of these features in present claim 1.
- 19.1 The board does not agree. The patent application discloses as the sole exemplified production system for bio-organic compounds that for isoprenoids (see e.g paragraphs [0022], [0051], [0054], [0092], claims 1, 7, 16, 21, 34 and 35). This, in the board's opinion, points to production systems for isoprenoids as preferred.

- 19.2 A concentration of "*at least 90% bio-organic compound*" in the organic second phase of the system is disclosed in claim 15 as filed, which likewise indicates that this feature is preferred. Since the production of isoprenoids as bio-organic compounds is likewise preferred, the patent application provides a direct and unambiguous basis for the combination of these two features in step d. of claim 1.
20. Secondly, the appellant submitted that the combination of "*genetically modified*" and "*bacterial or fungal host cells*" in step c. of claim 1 was obtained after performing "*several selection steps*".
- 20.1 The board again does not agree. Paragraph [0021] of the patent application mentions that the terms "*Host cell*" and "*microorganism*" are interchangeably used which refer "*to any archae, bacterial, or eukaryotic living cell into which a heterologous nucleic acid can be or has been inserted*" (emphasis added). In this context, paragraph [0083] of the patent application further discloses that any "*suitable host cell can be used in the practice of the present invention. In some embodiments, the host cell is a genetically modified host microorganism*" (emphasis added). In other words, both paragraphs disclose that genetically modified archae, bacterial or eukaryotic cells are an embodiment of the invention.
- 20.2 As regards eukaryotic host cells, the patent application mentions fungal cells only (see paragraphs [0088] to [0090]), which implies their preferred use as host cells in the patent application.
- 20.3 Examples of bacterial host cells are disclosed in paragraphs [0085] to [0087] of the patent application.

- 20.4 Furthermore, the working examples of the patent application disclose solely genetically modified bacterial and fungal host cells (see page 44, paragraph [0181] to page 80, first paragraph). This is a further indication that in the production system of claim 1 the use of genetically modified bacterial and fungal cells are preferred.
- 20.5 In view of these considerations, the board concludes that the host cells cited in step c. of claim 1 are indicated as preferred in the patent application, and hence, directly and unambiguously derivable therefrom.
21. Thirdly, the appellant submitted that the feature "*the organic second phase is spontaneously formed*" in step d. of claim 1 lacked a proper basis in the patent application.
- 21.1 Paragraph [0058] of the patent application states: "*In some embodiments, the organic second phase occurs spontaneously as a result of chemical and molecular interactions such as differences in solubility, or hydrophobicity, density, concentration or any other spontaneous phase separation mechanism. In other embodiments, separation of the first and second phases is induced in a separation vessel or vessels or system that may be the same or a different vessel or vessels or processing system as the fermentation vessel or vessels*" (emphasis added).
- 21.2 In other words, this paragraph teaches in general that the organic second phase forms either **spontaneously** or is **actively** separated from the aqueous first phase. Thus, this paragraph discloses two generic principles or alternatives to achieve a phase separation. A

selection of one generic alternative out of two does not constitute a selection from a list.

- 21.3 The appellant argued that the patent application disclosed various alternative mechanisms for separating organic and aqueous phases. However, all of the examples referred to by the appellant concern **actively induced** phase separation mechanisms (see page 12, last line to page 13, first paragraph of the patent application), i.e. these examples are embodiments of the second phase separation principle set out above, which is not cited in claim 1. In these circumstances, the appellant's argument does not convince the board.
22. Accordingly, the board concludes that claim 1, and hence, the main request complies with the requirements of Article 123(2) EPC.

Sufficiency of disclosure

23. The appellant submitted that the patent application lacked any information for the skilled person how in an industrial-scale production system a spontaneously formed organic isoprenoid phase could be separated from an aqueous phase. Example 23 of the patent application related to a hypothetical working example only. Although it mentioned settlement and decantation for separating an isoprenoid organic phase from the aqueous phase in a small-scale cultivation vessel ("250 mL"), it was silent on how decantation could be performed in an at least 100 l fermenter as cited in claim 1. Furthermore, Example 23 provided no information about the minimal isoprenoid titer to form an organic phase. Since the patent provided no information how to perform the claimed invention, the burden of proof to substantiate insufficiency by verifiable facts was not

on the appellant, but on the respondent. The respondent had not discharged their burden of proof by submitting the supplementary experimental data (see document D9 and D10), since these documents provided no evidence that the claimed invention could be carried out. The vessel sizes used therein were smaller than the size referred to in claim 1. Moreover, the data was obtained from a single experimental set-up, and hence, not sufficient to support the whole breadth of the claim. The required 24 hours for obtaining an organic layer was also too long for a spontaneous phase separation.

24. According to the established case law the provision of experimental evidence in the patent application for a claimed effect is not a prerequisite for patentability, since it suffices that based on the data in the patent application, or from common general knowledge it is plausible that a product (here an isoprenoid of a certain minimal length and concentration) is suitable for the claimed technical effect (here the spontaneous formation of an organic phase) (see Case Law of the Boards of Appeal of the EPO, 9th edition 2019, (hereinafter "Case Law"), II.C.7.2.).
25. The claimed invention, as set out above, is directed to a system for the production of isoprenoids of a minimum length by using genetically modified microorganisms in an aqueous culture utilising a carbohydrate as carbon source, which at an undefined point in time during the fermentation spontaneously form an organic phase of at least 90% isoprenoids.
26. The patent application discloses in paragraph [0058] that the spontaneous formation of an organic phase in an aqueous phase is the "*result of chemical and molecular interactions such as differences in*

solubility, or hydrophobicity, density, concentration or any other spontaneous phase separation mechanism".

27. Due to their chemical nature, isoprenoids are hydrophobic, in other words, immiscible with water. In the board's opinion, the skilled person taking common general knowledge into account would therefore reasonably assume that isoprenoids due to their chemical properties form at a certain concentration inherently, and hence "*spontaneously*", an organic phase in an aqueous medium.

28. Example 23 of the patent application "*describes the production of linalool, limonene, β -pinene, β -phellandrene, carene, or sabinine in Escherichia coli host strains*", i.e. of various isoprenoids with at least 10 carbon atoms, in a bacterial host (see paragraph [0319]). A genetic modification of the *E. coli* cells used in Example 23 is implied by the addition of "*IPTG*" (a known inducer of heterologous gene expression) into "*250 mL*" culture vessels to start the production of isoprenoids. The isoprenoids are separated from the culture medium *inter alia* "*by settling and decantation if the titer of the compound of interest is large enough to saturate the media and to form a second phase*" (see paragraph [0321]).

29. A simple decantation of the organic phase by using, for example, taps in the vessel is disclosed in paragraph [0059] of the patent application.

30. In the board's opinion, based on the information in the patent application and the considerations indicated above, the skilled person would consider it at least plausible that isoprenoids produced by microbes and secreted into the aqueous medium spontaneously form an

organic phase, depending on their concentration. Moreover, since this is an inherent property of isoprenoids in water, it occurs independently of the vessel or sample size used.

31. It is further established case law that an effect which is at least rendered plausible by the teaching of the patent application can be backed-up by supplementary experimental evidence (see Case Law, II.C.6.8.). Documents D9 and D10 disclose two experiments using a genetically modified yeast cell for the production of farnesene, i.e. an C₁₅-isoprenoid (see "*Experimental Summary*"). After a settling time of 24 hours, the culture samples form an organic layer of farnesene that overlays the culture medium (see documents D9 and D10, Figures, and document D11, point 4). Further, the respondent submitted a report of an integrated biorefinery pilot project that started on 28 December 2009 (see document D8, front page). Document D8 discloses a pilot plant that houses "*two 300L fermentors*" (see page 8, first paragraph, below the heading "*Task A: Pilot plant upgrades and operations*"), and mentions that the purity of crude farnesene using various sugar syrups as carbon source for fermentation is consistently above 90% (see page 38, Table A.7). The appellant submitted that the crude farnesene did not spontaneously form but had been separated from the medium by centrifugation (see page 37). The board does not agree, since the centrifugation step described on page 37, last paragraph to the first paragraph on page 38 of document D8 concerns the clarification of the sugar syrup used as carbon source prior to sterilization and fermentation.
32. The appellant further submitted that the supplementary data did not demonstrate that the claimed production

system could be put into practise. The board does not agree. Although documents D9 and D10 use cultivation vessels that are smaller than the minimum size of 100 l cited in claim 1, and use samples of the fermenter medium instead of the whole medium to demonstrate the spontaneous formation of an isoprenoid organic phase by gravity settlement, the board has no doubts that these experiments are representative for the claimed production system, since as set out above, isoprenoids at a certain concentration spontaneously form an organic phase as an inherent property in an aqueous medium, irrespective of the vessel size or sample size used. Moreover, the spontaneous formation does not impose any time restrictions.

33. The appellant has not submitted any evidence of non-working embodiments falling within the scope of claim 1, or other verifiable facts in support of doubts that the claimed system could be carried out across the whole breadth of claim 1. In *inter partes* proceedings, the burden of proof initially rests upon the opponent to establish, based on the balance of probabilities, that a skilled person reading the patent application/patent using his/her common general knowledge would be unable to carry out the invention. The burden of proof can be reversed, however, when the patent application/patent does not contain detailed information of how to put the invention into practice, and hence, a weak presumption exists that the invention is sufficiently disclosed. It is then up to the patent proprietor to prove the contrary (see Case Law, II.C.9.).

34. As set out above, the board is convinced that based on the information provided in the patent application, it is at least plausible that the microbial production of isoprenoids with the properties as defined in claim 1

results in the spontaneous formation of an organic phase in an aqueous medium. In the absence of any evidence to the contrary, the board concludes that the main request complies with Article 83 EPC.

Novelty

35. The appellant submitted that the subject-matter of claim 1 lacked novelty over documents D1 to D3.
36. As set out above, the board does not agree with the appellant that the "fermenter size" feature of claim 1 relates to an arbitrary design option, but is a structural feature of the claim. Since, the fermenters/cultivation vessels in documents D1 to D3 have sizes of "10-L", "1.5 l", or of "40 ml"/"125 ml", respectively (see page 685, column 2, second paragraph of document D1; page 720, column 2, third paragraph of document D2; column 3, lines 24 and 25, and column 4, lines 5 to 7 of document D3), which are all smaller than 100 l, the board concludes that the subject-matter of claim 1 is novel over the cited documents, at least because of this feature.
37. Accordingly, the main request complies with the requirements of Article 54 EPC.

Inventive step

Closest prior art and technical problem

38. The appellant selected either documents D1, D2 or D3 as closest prior art in combination with document D5, or alternatively document D6 as closest prior art combined with documents D1, D2 or D3. The respondent selected document D1.

39. According to established case law, a document aiming at the same purpose or effect and having most of the relevant technical features in common with the claimed invention normally represents the closest prior art (see Case Law, I.D.3.1).
40. Document D1 discloses a method for the production of amorpha-4,11-diene (a C₁₅ isoprenoid) by an engineered *E. coli* strain using a two-phase partitioning bioreactor (TPPB) (see abstract). The cells are grown on glycerol as carbon source (see page 685, column 2, first paragraph). Although glycerol is a polyol, it is commonly used in foods as sweetener, and hence, in the present case can be regarded as a carbohydrate. The bioreactor in document D1 has a volume of "10-L" (see page 685, column 2, second paragraph). Although the first sentence in the abstract of document D1 mentions "*large-scale fermentations*", this statement does not relate to the production process or the system actually disclosed in document D1, but rather indicates a general technical context. Document D1 further mentions that an overlay of the organic solvent dodecane is added to the fermenter vessel for trapping volatile amorpha-4,11-diene. The dodecane phase contains more than 97% of the generated amorpha-4,11-diene (see page 686, column 1, first paragraph, page 687, column 1, second and third paragraphs, page 690, column 1, third paragraph, column 2, second paragraph). Alternatively, volatile amorpha-4,11-diene is isolated by condensation in a cold trap cooled with dry ice and ethanol (see page 686, column 1, second paragraph). The appellant submitted that a spontaneous formation of an organic phase related to any phase separation mechanism, including the use of a cold trap. Therefore no difference existed between the organic phase obtained

by the cold trap in document D1 and claim 1. The board does not agree. While an "*organic second phase*" that "*is spontaneously formed*" as cited in claim 1 without further specifications indeed relates to organic phases that form without an external activity (see above) in any physical state, claim 1 further defines that the organic phase spontaneously formed is "*liquid*" and "*in contact with the first [aqueous] phase*". However, the amorphous-4,11-diene in document D1 is volatile, and moreover, no longer in contact with the aqueous phase.

41. In summary, the subject-matter of claim 1 differs from document D1 in the "fermenter size" feature (100 l vs 10 l), and in the "organic phase" feature (no disclosure that the organic phase consists of at least 90% isoprenoid, and is in contact with the aqueous phase).

42. Document D2 discloses an aqueous-organic two-phase system for the bacterial conversion of carveol (an isoprene) into the C₁₀ isoprenoid carvone by using *Rhodococcus erythropolis* (see title and abstract; also document D3, Figure 1). Document D2 is silent on whether this *Rhodococcus* strain is genetically modified, or non-modified (see page 720, column 1, fourth and fifth paragraph, column 2, second paragraph). The fermenter in document D2 has a "*working volume of 1.5 l*" (see page 720, column 2, third paragraph). Different carbon sources are used in the growth medium for optimising the conversion reaction, including an C₁₀ isoprenoid ("*limonene*"), alcohols such as "*ethanol*", or "*cyclohexanol*", an alcoholic derivative of cyclohexane (i.e. a cycloalkane) (see page 720, column 2, third paragraph). None of them is a carbohydrate. The bacterial conversion of carveol into carvone is either performed in a single-aqueous phase

system (see page 720, column 2, last paragraph), or in a two-phase system by adding an overlay of "iso-octane" or "n-dedecane", i.e. two organic solvents (see page 721, column 1, second paragraph). The one-phase system adds "ethylacetate" at a later stage to extract the carvone from the aqueous phase (see page 720, column 2, last paragraph, and page 721, column 1, fourth paragraph).

43. Accordingly, the subject-matter of claim 1 differs from document D2 in the "fermenter size" feature (100 l vs 1.5 l), the "aqueous phase" feature (no genetically modified bacteria, no carbohydrate as carbon source), and the "organic phase" feature (no disclosure that the organic phase comprises at least 90% isoprenoid).

44. Document D3 discloses a genetically modified *E. coli* that is used in a two-phase aqueous-organic system for producing various isoprenoid derivatives (α -terpineol, carveol, carvone, and perillyl-alcohols) of the C₁₀ isoprenoid limonene (see column 1, lines 10 to 20, 26, 42, column 2, lines 51 and 52, and Figure 1). The isoprenoid derivatives are extracted from the aqueous medium as soon as they are formed by adding an overlay of an organic solvent, preferably limonene (see column 1, lines 10 to 17, column 2, lines 47 to 50, column 5, lines 5 to 14, and Figure 3). Aside its function for extracting the derivatives, limonene is also used as carbon source for the *E. coli* cells to produce the derivatives (see column 2, lines 47 to 51). Document D3 uses "40 ml serum bottles" or "125 mL screw cap bottles" as cultivation vessels (see column 3, lines 24 and 25, and column 4, lines 5 to 7, respectively).

45. Thus, the subject-matter of claim 1 differs from document D3 in the "fermenter size" feature (100 l vs

40/125 ml), and the "aqueous phase" feature (no carbohydrate as carbon source). Furthermore, since in document D3 an isoprenoid serves as extraction medium and carbon source, the two-phase system is based on a concept different from the claimed production system.

46. Document D6 discloses a further two-phase aqueous-organic system for the production of β -carotene, a C₄₀ isoprenoid, by the microalgae *Dunaliella salina*. Since β -carotene is intracellularly produced in chloroplasts and not secreted into the medium, alkene-based organic solvents, including decane and dodecane, are added to the medium for its extraction from the cells (see abstract, and Figure 3). The cells are cultivated in 50 ml growth medium (see page 178, column 2, fourth paragraph), which implies the use of a fermenter that is significantly smaller than 100 l. Document D6 is further silent on the use of genetically modified organisms, let alone a bacterium or a fungus, including the use of carbohydrates as carbon source. Furthermore, since β -carotene is extracted from the cells by adding an organic solvent, document D6 does not unambiguously disclose that the organic phase comprises at least 90% β -carotene and 10% or less of the alkene solvent.
47. In summary, document D6 differs from the claimed subject-matter in the "fermenter size" feature, the "aqueous phase" feature (no genetically modified bacteria or fungi, no carbohydrate as carbon source), and the "organic phase" feature (no disclosure of an organic phase formed of at least 90% isoprenoid).
48. Consequently, documents D1 to D3 and D6 are all directed to the same purpose underlying the claimed subject-matter, namely the provision of a biotechnology-based isoprenoid production system. The

system disclosed in document D1 shares most of the relevant technical features with the claimed subject-matter (since it differs in two features only), while documents D2 and D6 differ in three features, and document D3 relates to a different concept (use of an isoprenoid as extraction medium and carbon source).

49. In line with the case law (see above) the board considers that document D1 represents the closest prior art. The larger vessel size of the claimed system enables the microbial production of isoprenoids in higher amounts. The direct use of a spontaneously formed organic phase for separating isoprenoids from the aqueous phase instead of adding an organic solvent or using a cold trap results in the provision of a simplified production system, since less reagents/technical means and process steps are required.
50. The technical problem to be solved is thus defined as the provision of a simplified production system for isoprenoids.
51. The board is convinced that this problem is solved by the subject-matter of claim 1 in light of Example 23 and paragraph [0048] of the patent.

Obviousness

52. It remains to be assessed whether or not the skilled person starting from document D1 and faced with the technical problem identified above, would have arrived at the claimed production system in an obvious manner.
53. Document D1 explicitly suggests the use of large-scale fermentations (see abstract), which obviously implies the use of larger fermenter vessels, including for

example, a volume of 100 l. Accordingly, the distinguishing "fermenter size" feature alone does not render the claimed production system inventive over document D1. Document D5 only discloses that large-scale aerobic fermentation reactors are known since the 1930s (see page 167, column 1, second paragraph), and that such fermenters are applicable for fermentation volumes from 1 L to several 1000 L (see page 168, column 2, last paragraph, and Tables 7.2 and 7.3 on pages 169 and 170). Therefore, none of documents D1 to D3, D5, or D6 suggest or hint at the spontaneous formation of an organic phase of microbially-produced isoprenoids during fermentation. Nor that this property of the isoprenoids could be exploited for directly separating an isoprenoid phase from an aqueous cultivation phase, and hence be useful in the provision of a simplified production system.

54. Accordingly, the subject-matter of claim 1 is not obvious in the light of the teaching of document D1 alone, or in combination with document D5. The same applies for the combination of any of the other documents cited by the appellant.
55. Thus, the main request complies with Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairman:



L. Malécot-Grob

B. Stolz

Decision electronically authenticated