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Datasheet for the decision of 27 April 2021

Case Number: T 0852/17 - 3.3.08

Application Number: 07863106.6

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Language of the proceedings: EN

Title of invention:
CONDITIONING BIOMASS FOR MICROBIAL GROWTH

Patent Proprietor:
Danisco US Inc.

Opponent:
Novozymes A/S

Headword:
CONDITIONING BIOMASS FOR MICROBIAL GROWTH/DANISCO

Relevant legal provisions:
EPC Art. 100(c), 123(2), 83, 54, 56, 84
RPBA 2020 Art. 12(3)
RPBA 2007 Art. 12(4)

Keyword:
Admission of new documentary evidence (yes);
Main request - added matter (yes)
Twelfth auxiliary claim request (ACR12) - Novelty (no)
Thirteenth, Fourteenth, Seventeenth and Eighteenth auxiliary claim requests (ACR13; ACR14; ACR17; ACR18) - Inventive step (no)
Nineteenth auxiliary request (ACR19) - admission (no)

Decisions cited:
T 1852/11, T 1201/14, T 1525/17, T 0962/98, T 1906/11

Catchword:

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Case Number: T 0852/17 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 27 April 2021

Appellant: (Patent Proprietor)	Danisco US Inc. 925 Page Mill Road Palo Alto, California 94304 (US)
Representative:	Ward, Eliot Mewburn Ellis LLP Aurora Building Counterslip Bristol BS1 6BX (GB)
Appellant: (Opponent)	Novozymes A/S Krogshoejvej 36 2880 Bagsvaerd (DK)
Representative:	Didmon, Mark Potter Clarkson The Belgrave Centre Talbot Street Nottingham NG1 5GG (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 31 January 2017 concerning maintenance of the European Patent No. 2099899 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	D. Rogers

Summary of Facts and Submissions

I. European patent N° 2 099 899 based on European patent application N° 07863106.6 hereinafter "the patent application" was opposed on the grounds of Articles 100 (a), (b) and (c) EPC. An opposition division considered that the main request and auxiliary requests 1 and 2 failed to meet the requirements of Articles 123(2), 54 and 56 EPC, respectively and decided to maintain the patent on the basis of auxiliary request 3.

II. The patent proprietor (appellant I) lodged an appeal against the decision of the opposition division. It submitted with its statement of grounds of appeal the same set of auxiliary requests ACR1 to ACR8 and the ninth to eleventh auxiliary requests underlying the decision under appeal. In reply to the appellant II's statement of grounds of appeal, it filed further twelfth to nineteenth auxiliary requests (ACR12 to ACR19).

III. The opponent (appellant II) lodged an appeal against the decision of the opposition division and submitted documents D17 and D18.

IV. The parties were summoned to oral proceedings. In a communication pursuant to Article 17(1) RPBA 2020, the parties were informed of the board's provisional, non-binding opinion, inter alia on issues concerning Articles 123(2), 83, 54 and 56 and Rule 80 EPC.

V. Oral proceedings were held by video conference on 27 April 2021 in the presence of both parties. At the end of these proceedings, appellant I withdrew auxiliary requests ACR1 to ACR11, ACR15 and ACR16.

VI. Claims 1 to 3 of the main request read as follows:

"1. A method for conditioning a lignocellulose biomass for use as a feedstock for a production microorganism that produces a desired protein, said method comprising providing a composition comprising a lignocellulose biomass, contacting said composition with a phenol oxidizing enzyme for a period of time sufficient to condition said composition, wherein the rate of growth of said production microorganism cultured in said conditioned composition is increased relative to that of a composition comprising lignocellulose biomass that was not contacted with said enzyme, and wherein said lignocellulose biomass comprises lignocellulose biomass that has not been subjected to acid pretreatment.

2. A method for culturing a production microorganism that produces a desired protein, said method comprising contacting a composition comprising a lignocellulose biomass with a phenol oxidizing enzyme for a period of time sufficient to condition said composition for microbial growth, and growing said microorganisms in said conditioned composition, wherein the rate of growth of said microorganism cultured in said conditioned composition is increased relative to that of a composition comprising lignocellulose biomass that was not contacted with said enzyme, and wherein said lignocellulose biomass comprises lignocellulose biomass that has not been subjected to acid pretreatment.

3. A method for producing a desired protein, said method comprising culturing a microorganism that produces said protein in a composition comprising lignocellulose biomass that is conditioned for microbial growth, wherein said conditioned composition

has been contacted with a phenol oxidizing enzyme for a period of time sufficient to condition said composition, and wherein the rate of growth of said microorganisms cultured in said conditioned composition is increased relative to that of a composition comprising lignocellulose biomass that was not contacted with said enzyme, and wherein said lignocellulose biomass comprises lignocellulose biomass that has not been subjected to acid pretreatment."

Dependent claims 4 to 14 define specific embodiments of claims 1 to 3.

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

D1: WO2004/029193 (published 8 April 2004);

D2: WO2005/005646 (published 20 January 2005);

D3: WO2005/074656 (published 18 August 2005);

D4: WO2008/134259 (published 6 November 2008);

D5: M. Bigelow, and C.E. Wyman "Cellulase production on bagasse pretreated with hot water." Applied Biochemistry and Biotechnology, vol. 98, pp. 921–934 (2002);

D8: S. Larsson, *et al.*, "Comparison of Different Methods for the Detoxification of Lignocellulose Hydrolyzates of Spruce." Applied Biochemistry and Biotechnology, vol 77, pp. 91–103 (1999);

D13: USDA National Nutrient Database for Standard Reference Release 27, Basic Report 2014, Corn grain, yellow;

D14: D.E. Akin and L.L. Rigsby, "Corn Fiber: Structure, Composition, and Response to Enzymes for Fermentable Sugars and Coproducts." Appl Biochem Biotechnol, vol. 144, pp. 59–68 (2008).

D15: M. Gáspár, *et al.* "Corn fiber as a raw material for hemicellulose and ethanol production", Process Biochemistry, vol. 42 (7), pp. 1135-1139 (2007);

D16: C. Martin, *et al.* "Ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilising Saccharomyces cerevisiae" Enzyme and Microbial Technology, vol. 31, issue 3, pp. 274-282 (2002);

D17: R.M. Vohra, *et al.* "Effect of Lignin and Some of its Components on the Production and Activity of Cellulase(s) by Trichoderma reesei." Biotechnology and Bioengineering, vol. 22, pp. 1497-1500 (1980).

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

Main request
Article 100(c) EPC

The term "desired protein" derived from paragraphs [0052], [0057] and [0058] and claims 11 and 12 of the patent application. Decisions T 962/98 of

15 January 2004 and T 1906/11 of 18 January 2013 considered that an intermediate generalisation was allowable if it was the result of unambiguous information that the skilled person would have drawn from a review of the content of the application as filed (see Case Law of the Boards of Appeal of the European Patent Office, Ninth Edition, July 2019; Chapter II.E.1.9).

The amendments "for use as a feedstock", "has not been subjected to acid pretreatment" and that the desired protein was an enzyme or cellulase in dependent claims 11 and 12 complied all with the requirements of Article 123(2) EPC.

Twelfth auxiliary claim request (ACR12)
Article 123(2) EPC

The amended methods of claims 1 to 3 were based on claim 11 referring back to independent claim 3 of the patent application while claims 1 and 2 were based on the methods of claims 1 and 2 and the teaching of [0058] referring to the production of microorganisms of the patent application.

The introduction of a specific reference to a "phenol oxidizing" enzyme at the end of claims 1 to 3 was intended to clarify that the phenol oxidizing enzyme was not the "desired enzyme" produced by the production microorganism. This clarification was directly occasioned by the first amendment introduced in granted claims 1 and 3.

Sufficiency of disclosure (Article 83 EPC)

The findings and the reasons developed in item 7.4.3 of the decision under appeal were adopted by appellant I.

Novelty (Article 54 EPC)

None of the documents D1 to D4 anticipated the claimed subject-matter. Reference was made to the arguments put forward in opposition proceedings as to why documents D3 and D4 were not novelty destroying.

As claims 1 to 3 related to methods, reference was made to the Guidelines G-IV 7.2.1 and decision G2/88. Although the claims were drafted as method claims, whether an activity was claimed as a method of carrying out an activity (setting out a sequence of steps) or as the use of a thing for a stated purpose (the sequence of steps being implied) was merely a matter of preference: With regard to the relevant Enlarged Board decisions, there was no difference in substance. Since claims 1 to 3 related to the use of a phenol oxidising enzyme for the purpose of conditioning a lignocellulose biomass composition, causing the lignocellulosic inhibitory effect in the biomass to be reduced, the growth rate of production microorganisms subsequently cultured in said conditioned lignocellulose composition was increased. This increased growth rate was accordingly a functional technical feature of the claims (see G 2/88 third headnote and point 10.2 of the reasons).

Document D1 disclosed the use of laccase to aid fermentation by depleting oxygen and oxidising the inhibitory compounds (see page 3, lines 6-12), and focused on methods relating to fermentation and using fermenting microorganisms for ethanol production (see abstract; claims 26 and 27). Even if a laccase was

added to a biomass composition in Example 14 which consisted of dry milled yellow corn, the rate of microorganism growth cultured on the composition was not measured and could not be inferred from the information disclosed (see D1, Table 10; patent [0057]). There was no disclosure in document D1 of a method of production of a desired protein or of culturing a production microorganism producing a desired enzyme. Corn fiber was known to be a by-product of the corn wet-milling industry and its major component was the pericarp consisting of 35% hemicellulose, 18% cellulose and 20% remaining starch. It was hypothetical that dry-milling of corn would result in the same components, including lignin. Any conclusion regarding the necessary use of lignocellulosic material and regarding the growth of microorganism from the ethanol production data in example 14 had to be excluded. Thus, the subject-matter of claims 1 to 3 was novel over document D1.

Document D2 described the use of fatty acid oxidizing enzymes to improve fermentation in an ethanol production process (see abstract) and stated that "...the fermentation process is used in combination with a liquefaction process and/or saccharification process, in which additional enzymatic activities, such as esterase, including lipase and/or cutinase; phytase; laccase; cellulase: xylanase; alpha-amylase; glucoamylase; or mixtures thereof, may be used for processing the substrate, e.g., a starch substrate." (see page 4, lines 24-28 and page 15, lines 12-36).

Document D3 related to isolated cellulase enzymes (termed cellulolytic proteins) for improved cellulose degradation and a method for producing an organic

substance comprising: (a) saccharifying a cellulosic material with an effective amount of a cellulolytic protein in the presence of an effective amount of the polypeptide having cellulolytic enhancing activity of claim 8, ... (b) fermenting the saccharified cellulosic material of step (a) with one or more fermentating microorganisms; and (c) recovering the organic substance from the fermentation (see claims 53 to 61). The cellulosic material was not necessarily lignocellulose (see document D3, claim 53, p. 4, second full paragraph, last sentence).
Hence, it lacked a clear and unambiguous disclosure of a double selection combining laccase enzymes, as additional enzyme(s), with a lignocellulose as the cellulosic material and failed to disclose the production of a desired enzyme by fermenting microorganisms too (e.g. claims 60 and 61).

Since documents D2 to D4 failed to disclose the technical effect of increased microorganism growth through the use of phenol oxidising enzymes in the "pre-treatment", their content could not deprive the claimed subject-matter of novelty (see patent [0017], last two sentences; [0023], first two sentences).

Document D16 described an ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilising Saccharomyces cerevisiae. S. cerevisiae TMB 3001 produced ethanol from both detoxified samples, whereas a 2 hours lag was observed in the fermentation of the undetoxified hydrolysate (Fig. 4). The rate of ethanol formation was slightly faster for the overlimed- than for the laccase-treated hydrolysates. The effect of detoxification and the ethanol production during fermentation of the recombinant xylose-fermenting S. cerevisiae strain TMB

3001 in bagasse hydrolysates was analysed under anaerobic conditions. The results obtained under these conditions did not allow to conclude whether these same conditions had also an effect on the growth rate of the microorganism. Hence, document D16 provided no information that the culture conditions and media selected for increased ethanol production would inevitably lead to an increased growth rate of the cultured production microorganism. There was neither an explicit nor an implicit disclosure in document D16 of a production microorganism producing a desired protein as defined in claims 1 to 3.

Thirteenth auxiliary claim request (ACR13)
Articles 54, 83 and 123(2) EPC

The previous submissions with regard to Articles 54, 83 and 123(2) EPC were maintained.

Admission of document D17 (Article 12(4) RPBA)

No reason was provided why document D17 could not have been filed earlier e.g. during opposition proceedings. A reference to *Trichoderma* existed in Auxiliary request 3 and in granted claim 9 filed prior to the oral proceedings. Since document D17 was prima facie no more relevant than any of documents D1 to D15 on file, especially not documents D5 and D8, there was no reason to admit it into the appeal proceedings.

Inventive step (Article 56 EPC)

Document D5 represented the closest prior art. It related to the growth of cellulase-producing *T. reesei* microorganisms on a lignocellulosic biomass (bagasse), which had not been acid pretreated (see title). Various

growth curves and problems with a lag in the microorganism growth were reported and postulated to be due to the lignin present in the biomass (see Figures 1-5; page 927, second paragraph). The results of filtration studies showed that the inhibition of cultures grown on filter-sterilized hydrolysate was much lower, as the filtering process apparently removed toxins or those constituents that become toxic in the autoclave, whereas no growth was observed when Rut C30, after having been adapted on a medium comprising 30% hydrolysate for 12 days, was transferred to a medium comprising 80% hydrolysate (see Figures 2 and 3; page 926, first paragraph). Document D5 concluded that "more work is needed to pinpoint what caused the lag ... " (see page 933 line 3). It showed that heavy washing of the biomass was required to achieve "good growth and cellulase production rates" (see lines 9-10 of the abstract). The methods for reducing growth inhibition achieved only limited success (see page 927, lines 13 and Figures 4 and 5).

The difference between document D5 and the method of claim 1 was that the lignocellulosic biomass was conditioned by a phenol oxidizing enzyme.

The effect underlying this difference was that dramatic cell growth of the laccase treated culture was observed by means of its glucose consumption (see Figure 1: 0.5/2 day and 0.5/3 day). No cell culture lag was observed.

The solution was the method of claim 1.

In view of the content of document D5, the technical problem was to improve growth of cellulase-producing

microorganisms on a non-acid-pretreated lignocellulose biomass.

Although document D5 identified lignin itself (or its solid components) as the inhibitor(s), the abstract states: "Adding lignin to Solka Floc delayed enzyme production, suggesting that lignin or other materials in the lignin solids could cause the lag observed for pretreated bagasse, but more studies are needed to resolve the actual reason for this delay" (see final sentence).

Document D5 suggested nowhere that phenolic compounds inhibited cellular growth. Thus, the skilled person would not try to neutralise any one of these classes of phenolic compounds in order to improve the growth characteristics of cellulase producing microorganisms by using a phenol oxidising enzyme such as laccase.

Document D8 proposed twelve different detoxification treatments, one of which used *Trichoderma reesei* itself as a "detoxifying" treatment for *S. cerevisiae* (see page 97, lines 12). In this context, the patent noted that *Trichoderma* species produce laccase (see paragraph [0039]). Hence, the skilled person would not expect laccase treatment of a lignocellulose biomass to benefit this species of cellulase-producing microorganism. Since documents D5 and D8 used different cells - i.e. Trichoderma, which produces its own laccase, and S. cerevisiae - and different pretreated type of lignocellulosic biomass, the skilled person had no reason to combine their teaching. Treatment with laccase affected only the concentration of phenolic compounds and was the only method that was specific for one group of inhibitors (see page 101, last sentence). Ethanol productivity and the biomass yield were only

slightly lower than in the reference fermentation (47% and 61%, respectively), while the treatment with anion exchange at pH 10 removed most of the furan derivatives, probably owing to hydrophobic interactions with the anion-exchange matrix, resulting in higher productivity and biomass yield (see page 102, lines 23-26).

Thus, the skilled person would not select laccase pretreatment as an obvious solution to improving the growth of the cellulase-producing microorganisms of document D5.

Similarly, document D16 described that the use of a hydrolysate detoxified by overliming resulted in a higher ethanol yield during fermentation than a hydrolysate detoxified by laccase treatment (see Fig.4; page 278, col.2, lines 14-16).

In conclusion, there was no clear teaching in the art that the use of phenol oxidising enzymes in general, nor a laccase in particular, enhanced microorganism growth on a non-acid pretreated lignocellulose biomass.

Fourteenth auxiliary claim request (ACR14)

Basis for the amendment was paragraph [0014] and original claim 10. The amendment was responsive to both the novelty and inventive step objections.

Inventive step (Article 56 EPC)

The arguments submitted in respect of the thirteenth auxiliary claim request (ACR13) were maintained.

First, there was no disclosure in document D5 that phenolic compounds were inhibitors to *Trichoderma*. Second, document D5 showed that *Trichoderma* Rut C30 did not grow on 80% conditioned hydrolysates (see Fig.3), which rendered the use of *Trichoderma* producing laccase, or the use of laccase itself, to achieve a detoxification of the lignocellulosic biomass more than questionable.

In this context, document D17 reported an effect on the production of cellulases of *T. reesei* when cultured in the presence of three monomer model compounds: vanillin, protocatechuic acid and ferulic acid (see abstract; p.1498, lines 13-16). The concentrations tested of lignin and monomer model compounds were identical: 0.01, 0.03, and 0.05% (w/v) (see p.1497, lines 27-29). This amounted to an over-representation of the concentration of said monomer model compounds in relation to their naturally occurring concentration. This artificially high concentration of vanillin in document D17 stood in contrast to the concentration of 0.12 g/L (corresponding to 0.012% (w/v)) vanillin determined in the dilute acid hydrolysate of spruce (see document D8, Table 1). Vanillin represented 0.05% in w/w of steam exploded poplar (see document D10, page 568, Table 2). The fungal biomass decreased from 80 mg mycelial dry weight to 47 mg/flask when cultured in presence of 0.01% lignin while it was only reduced to 73 mg/flask when cultured in presence of 0.01% vanillin (see page 1498, lines 13-16; page 1497, lines 1-5, Fig. 1(a) and (b)).

Since document D5 related to a method of fermenting *S. cerevisiae* under anaerobic conditions for producing ethanol, while the claimed method required the culture of *Trichoderma* for increasing its growth rate, which is

further distinguished from *S. cerevisiae* by its biology, it was definitely inventive to transfer a phenol oxidizing enzyme pretreatment of a lignocellulosic biomass from the method described in document D8 to the method described in document D5.

Seventeenth auxiliary claim request (ACR17)

Basis for the amendment was in paragraphs [0028], [0032] and original claim 7. The amendment was responsive to the inventive step objections.

Inventive step (Article 56 EPC)

The arguments submitted in respect of the fourteenth auxiliary claim request (ACR14) were maintained.

The method of claim 1 and of document D5 differed in that the pretreated lignocellulosic biomass was corn stover. Chemical, physical, and biological methods to remove inhibitors were known to increase the fermentability of the substrate before fermentation. However, the effect of the different methods could not be compared with each other because different hydrolyzates and different microorganisms were used in the recited prior art fermentations (see document D8, page 92 line 35 to page 93 line 12). The results obtained by different pretreatment methods could not be directly transposed, a prohibition which must apply to the results obtained by the method of document D8 to the method of document D5.

Eighteenth auxiliary claim request (ACR18)

Basis for the amendment was original claim 13 and paragraphs [0079]-[0080] of the examples. The amendment was responsive to the inventive step objections.

Inventive step (Article 56 EPC)

The arguments submitted under Article 56 EPC for the seventeenth auxiliary claim request (ACR17) were repeated. The method of claim 1 using a phenol oxidizing enzyme had to comprise a laccase produced by a first *Trichoderma* species or a *Stachybotrys* species, and the microorganism cultured in said composition was a second *Trichoderma* species. None of the cited documents disclosed or suggested such a configuration, even less within the context of conditioning a corn stover biomass.

Nineteenth auxiliary claim request (ACR19)

The nineteenth auxiliary claim request (ACR19) corresponded to the twelfth auxiliary claim request (ACR12), but with claims 1 to 3 further specifying that the lignocellulose is un-pretreated.

Admission of the nineteenth auxiliary request into the appeal proceedings

The nineteenth auxiliary claim request (ACR19) was submitted with the reply to appellant II's statement of grounds of appeal, taking into account an objection raised under Rule 80 EPC. It corresponded to the eighth auxiliary claim request (ACR8) filed in opposition proceedings.

IX. The submissions made by the **appellant II**, insofar as relevant to the present decision, are summarized as follows:

Main request
Article 100(c) EPC

Claim 1 referred to a "desired protein" instead of a "desired product" and for the reasons set out in the impugned decision infringed Article 123(2) EPC.

There was no direct and unambiguous disclosure of a method of claims 1 to 3 which "produces a desired protein [product]", or used a lignocellulosic biomass that was "not subjected to acid pretreatment", and even less for a method combining these features with the specific feature of the dependent claims. Thus, claims 4, 11 and 12 added subject matter.

Twelfth auxiliary claim request (ACR12)
Article 123(2) EPC

Each of the claims 1-3 were amended to include the term "enzyme". Claim 11 of the patent application was only dependent on independent claim 3 and disclosed as such a preference in relation to the method of claim 3. Amended methods of claims 1 and 2 referring to an "enzyme" combined with features of the dependent claims resulted in embodiments combining individual features selected from separate lists of the patent application for which there was no basis.

Sufficiency of disclosure (Article 83 EPC)

The method of claim 1 was insufficiently disclosed because no definition was provided in the patent on how

"to condition" a composition for microbial growth. The claim mentioned no test for determining whether conditioning had occurred, let alone for determining a sufficient period of time for a conditioning to occur. Thus, a skilled person was incapable of performing the claimed invention, contrary to the requirements of Article 83 EPC.

The patent mentioned also the use of a laccase from *Trichoderma* without indicating how said enzyme was obtainable, e.g. from T. *piluliferum* (see patent application on page 21, lines 10 to 11). Thus, the embodiment of claim 14 could not be carried out without undue burden.

Novelty (Article 54 EPC)

Claims 1 to 3 related to any "[a] composition comprising a lignocellulose biomass" which meant any matter comprising lignin and cellulose, such as corn, and wherein the second contacting step could occur for as long as desired, as no microorganism, no level of increase and no test to determine this increase was specified in these claims.

The decision under appeal decided correctly that the claimed subject-matter was anticipated by any of documents D3, D4 and D16, but incorrectly that documents D1 and D2 were not novelty destroying.

Document D1 described a method where laccase was applied during propagation of a production microorganism and promoted the oxidation of inhibitors (see page 3, lines 6-10). The use of a feedstock comprising lignocellulose biomass, such as a whole corn milled, corresponded to a composition comprising a

lignocellulosic biomass, which had been physically, but not acidicly, pretreated (see page 20, lines 22-24). Hence, a selection from one list was therefore enough to arrive at the claimed solution (see page 6, lines 8-14).
Example 14 described the use of dry-milled yellow corn as a feedstock for a fermenting microorganism, which was not acid-pretreated but was treated with laccase. Documents D13, D14 and D15 demonstrated that corn fibers contained lignocellulose.
The patent specified that an increase in the growth rate of a production microorganism could be ascertained by a number of different parameters, such as, but not limited to, nutrient consumption, catabolite accumulation, pH, cell mass, cell number, etc. Thus, if any one of these parameters reflected an increase in the growth rate, the effect of the method claimed was achieved (see patent, col.16, lines 35-39).

Document D2 related to enzymatic processes and compositions for producing fermentation products (see abstract). In particular, the fermentation process was used in combination with a liquefaction process and/or saccharification process, in which additional enzymatic activities, such as laccase was used for processing the substrate, e.g. a starch substrate (see page 4, lines 24 to 31). Any suitable substrate or raw material could be used in this fermentation process, such as starch-containing materials (see page 5, lines 21-31). The fatty acid oxidizing treatment could be further used in combination with laccase (see page 15, lines 12 to 19), whilst the preferred application of the fermentation processes was an ethanol production process, where the raw material was preferably dry-milled, and where the whole kernel was milled and used (see page 23, line 3).

Documents D3 and D4 and the present patent defined a lignocellulosic biomass as lignocellulose-containing materials (see documents D3, page 4, second full paragraph; D4, page 2, lines 20-22 and page 3, line 11; patent paragraph [0019]). Documents D3 and D4 disclosed a method for conditioning a lignocellulosic biomass using a laccase (see document D3, claim 57) and a method for conditioning a lignocellulosic biomass using a phenol oxidizing enzyme (see document D4, claim 1) with, as one of the possible pretreatments, a non-acid pretreatment, falling under the scope of claim 1 (see document D4, example 4 or list on the paragraph spanning pages 9 and 10).

Document D16 disclosed hydrolysates of sugarcane bagasse pre-treated by steam explosion at 205 and 215 °C, which were then hydrolysed with cellulolytic enzymes. The hydrolysates were detoxified by either phenol oxidase laccase, before being used as a substrate for fermentation into ethanol, or chemically by overliming. These hydrolysates were fermented with xylose-utilising Saccharomyces cerevisiae laboratory strain TMB 3001, which integrated and expressed heterologous recombinant D-xylose reductase (XR), xylitol dehydrogenase (XDH), as well as with S. cerevisiae strain ATCC 96581. The enzymatic treatment had a marked effect upon the removal of phenolic compounds: approximately 80% of the phenols were removed from both hydrolysate H205 and H215. The glucose of detoxified hydrolysates was readily fermented, whereas glucose still remained after 12 hours in undetoxified hydrolysates (page 277, col.2, lines 21-24; Fig. 1 and page 278 col.2, lines 1-4, Fig. 4). The specific productivity of the strain TMB 3001 in the detoxified hydrolysates was almost two-fold higher

compared to the one observed in undetoxified hydrolysates (see abstract, Table 3 and Figure 4).

Thus, documents D1 to D4 and D16 deprived at least claims 1 to 3 of novelty.

Thirteenth auxiliary claim request 13 (ACR13)
Articles 54, 83 and 123(2) EPC

The objections raised against the previous claim requests were maintained.

Novelty Article 54 EPC

Claim 1 referred to a "production microorganism" that produced a "desired enzyme". These expressions had to be interpreted in their broadest meaningful sense. Since, there was no requirement for the enzyme to have a particular function, to be heterologous or homologous to the microorganism, or to have a particular purpose (or not) in the microorganism and the method failed to contain a step of obtaining the enzyme after production, it was legitimate to interpret the "desired enzyme" to mean any enzyme produced by said production microorganism independent of its purpose.

Document D16 deprived claim 1 of novelty, because it disclosed a method of culturing S. cerevisiae cultured on lignocellulosic biomass for which an increased growth rate, based on the parameters in the patent, could be established when the lignocellulosic biomass was treated with laccase compared to one that was not.

Inventive step (Article 56 EPC)

Document D5 represented the closest prior art. It disclosed a culture of *Trichoderma* producing cellulase on hot water pretreated lignocellulose biomass (sugarcane bagasse). The presence of (an) inhibitory compound(s) was recognized and remedial actions were taken.

Document D5 recognized that pretreated biomass needed detoxification. It tested whether hot water pretreatment resulted in a lower need for detoxification. First, the liquid hydrolysate obtained after hot water treatment was less toxic to the growth of organisms than hydrolysate obtained after dilute acid pretreatment (page 922, middle of the bottom paragraph). Second, a series of cultures were grown on combinations of liquid hydrolysate from pretreated bagasse and 10 g/L of sorbitol as the carbon source, varying from 0 to 80% hydrolysate. A level of 40% hydrolysate delayed the initial cell growth (see page 924, last paragraph). Thus detoxification (in the shape of a wash step) was still required. The addition of lignin to Solka Floc delayed enzyme production. This suggested that lignin or other materials in the lignin solids could cause the lag observed for pretreated bagasse (see abstract; page 933, lines 7-9).

The difference between document D5 and the opposed patent was that a phenol oxidising enzyme was applied after the pretreatment instead of water washes.

The effect of this treatment was the reduction of the amount of soluble inhibitory phenolic compounds (see [0017]).

As no effect was actually shown in the patent that could be assigned to the pretreatment by phenol

oxidzing enzyme of a non-acid pretreated lignocellulosic biomass, the problem solved by the method of claim 1 provided at best a further method for culturing a production microorganism that produces a desired enzyme according to document D5.
As no data in the patent showed a cell culture lag phase, there was no need to address this issue. The experimental results established rather that the growth rate in a culture medium comprising a lignocellulosic biomass that had been conditioned by laccase was for a vast majority of treatments almost similar to the one that was not (see patent Figure 1).

The technical problem could therefore be formulated as the provision of an alternative method using less toxic (products of) pretreated lignocellulose biomass (suitable) for culturing an enzyme producing microorganism.

In the light of this technical problem, the skilled person would have combined the teaching of document D5 with document D8 concerned with the detoxification of spruce hydrolysates.

Document D8 disclosed that detoxification with laccase was one of the better methods to detoxify the lignocellulose hydrolysate and showed the best compromise between ethanol yield and loss of fermentable sugar (page 92, lines 28 to 34; pages 101 and 102 spanning paragraph). Even if the hydrolysate in document D8 was obtained by a dilute acid pretreatment and focused on detoxifying hydrolysate for ethanol production using *S. cerevisiae*, this fact would not have prevented the skilled person, using a non-acid pretreated biomass, from consulting the content of document D8 and to apply said teaching on document D5,

as research was more advanced in the fermentation field than in the close neighboring field of enzyme production (see patent [0010]).

Since the method of claim 1 was not limited to a particular type of lignocellulose biomass and microorganism, the skilled person would have combined the method of document D5, in an attempt to solve the technical problem identified above, by treating the hydrolysate with a phenol oxidizing enzyme used in document D8. This was one of the alternatives that the skilled person had to obviously select from.

Alternatively, the subject-matter of claim 1 lacked an inventive step based on document D5 in combination with document D16. Both methods used non-acid treated lignocellulosic biomass which needed to be detoxified.

Document D16 demonstrated that the removal of phenolic compounds by laccase treatment resulted in an increase in specific productivity of the production microorganism and implicitly the rate of growth of a microorganism (see abstract, Table 3 and Figure 4). Both laccase treatment and overliming proved to be efficient for detoxification of the bagasse hydrolysates. The enzymatic treatment selectively removed the phenolics, while overliming lowered the concentration of different types of inhibitors (see page 280, col.2 lines 3-7; lines 14-23). They were therefore capable of reducing toxicity.

The skilled person would have adopted a try-and-see attitude, in the sense that it would immediately have tested whether the expected effect was directly amenable when applying the detoxification step proposed in document D16 to the method of document D5.

Fourteenth auxiliary claim request (ACR14)
Articles 54 and 83 EPC

The objections raised against the previous claim requests were maintained.

Inventive step (Article 56 EPC)

The objections raised against the previous claim request were maintained. Alternatively, the subject-matter of claim 1 lacked an inventive step based on document D5 in combination with document D16 as well.

Document D5 provided the motivation for the skilled person to look for approaches for removing inhibitors from hydrolysates and would have led him to turn to the detoxification disclosed in document D8. The skilled person starting with document D5, using *Trichoderma* to determine whether pretreated substrates were suitable for cellulase production, faced with the technical problem identified above, would have combined its teaching with the one of document D8 to arrive at a "method" comprising *Trichoderma* as the production microorganism.

There was no factual evidence supporting appellant I's assertions that a saprophytic microorganism, such as *Trichoderma*, living on decaying wood, was not inhibited by compounds released during pretreatment of lignocellulosic biomass in the same way as S. *cerevisae*. There was no reasonable doubt that the laccase treatment in document D8 would be expected to be beneficial to the method in document D5. Thus, it was established that *Trichoderma* was not yet

sufficiently adapted to inhibitory compounds released during pretreatment of lignocellulose biomass.

Document D17 related to the effect of lignin and some of its components on the production and activity of cellulase production by *Trichoderma*, such as "Kraft lignin" - a non-acid pretreated lignin - and mediated by lignin derivatives monomeric components: vanillin, protocatechuic acid and ferulic acid, shown to inhibit growth and enzyme production by that microorganism (see Fig.1). The lignin monomers were known to be oxidized by laccase.

The skilled person starting with document D5 was motivated to investigate the compounds responsible for inhibiting the *Trichoderma* production microorganism and would have turned to document D17 and its solution consisting of a laccase treatment of the biomass for removing them.

Document D16 demonstrated that the removal of phenolic compounds by laccase treatment resulted in an increase in specific productivity of the production microorganism and implicitly in the rate of growth of a microorganism (see abstract, Table 3 and Figure 4).

Seventeenth auxiliary claim request (ACR17)

Inventive step (Article 56 EPC)

Even if document D5 and documents D8 or D16 related to different purposes and used different microorganisms, this was not decisive, as there were situations in which the skilled person, in an attempt to solve a technical problem already solved in a more advanced neighbouring technical field and prima facie applicable

to both fields, would turn to documents and their solutions to apply them in its own field with a reasonable expectation of success.

There was no reason why a different purpose of the method and different microorganisms would have deterred the skilled person from consulting these documents and their solutions to solve the problem at hand.

Document D17 identified that vanillin, a lignin monomer model phenolic compound in a biomass was capable of inhibiting *Trichoderma* and thus its growth rate (see page 1499, lines 1-5).

Document D5 referred to cellulosic biomass such as corn stover as low-cost feedstock for biologic production (see page 921, last paragraph). There was also no doubt that it was the lignin, when the lignocellulosic biomass was pretreated that produced inhibitors, such as phenolic derived lignin derivatives, resulting in a lower cellular growth rate. There were no results in the patent which made the pretreatment of corn stover, i.e. a particular lignocellulosic biomass substrate, by a phenol oxidizing enzyme surprising or remarkable. In consequence, the technical problem starting from the method of document D5 could at best be defined as the provision of a mere alternative method.

Eighteenth auxiliary claim request (ACR18)

Inventive step (Article 56 EPC)

No inventive activity could be acknowledged for a method for culturing a production microogranism, wherein the phenol oxidizing enzyme comprises a laccase produced by a first *Trichoderma* species or a

Stachybotrys species, and said microorganism cultured in said composition is a second *Trichoderma* species.
Indeed, when a microorganism produced an enzyme in insufficient quantity, then it was only logical for the person skilled in the art to supplement this lack of enzyme by adding an external quantity of enzyme.
Whether the enzyme was produced by another strain or not, which cannot be ascertained anyway by the wording "first" and "second" alone, is without effect on its activity. Even the patent considered them to be equivalent (see [0039]-[0042]).
Hence, this difference amounted to no more than an arbitrary choice from a number of different solutions, each of which were obvious to the skilled person (Case Law of the Boards of Appeal of the European Patent Office, Ninth Edition, July 2019, section I.D.9.19.8).

Nineteenth auxiliary claim request (ACR19)
Admission into the appeal proceedings

Claims 1 and 3 were not convergent and reinstated previously deleted claims 1 and 3.
This set of claims had never been discussed before, neither in writing nor during oral proceedings in opposition. Thus, it was doubtful that it addressed prima facie the inventive step objection raised against all the previous requests.

X. Appellant I requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request or alternatively on the basis of any of the auxiliary requests ACR12, ACR13, ACR14, ACR17, ACR18 and ACR19. It requested that documents D15 to D17 not be admitted into the proceedings.

XI. Appellant II requested that the decision under appeal be set aside and that the patent be revoked in its entirety and that document D17 be admitted.

Reasons for the Decision

Admission of documents D15 to D17 into the appeal proceedings

1. Appellant I objected to the admission of documents D15 and D16 into the opposition proceedings. Even if they were filed on or before the Rule 116 EPC deadline, they were filed after the 9 month opposition period. Since no explanations were offered for their late filing and their merits, they should not have been admitted into the opposition proceedings.

2. Documents D15 and D16 were admitted into the opposition proceedings (see decision under appeal, item 2.3). The board sees no reason to overturn the opposition division's decision to admit these documents into the proceedings. The EPC provides no legal basis for excluding, in appeal proceedings, documents which were correctly admitted into the first-instance proceedings (see decisions T 1852/11 of 10 October 2016, reasons 1.3; T 1201/14 of 9 February 2017, reasons 2). These documents are thus in the appeal proceedings.

3. Appellant I objected to document D17's admission, as no reason was provided why it could not have been filed earlier e.g. during opposition proceedings.

3.1 Pursuant to Article 12(4) of the Rules of Procedure of the Boards of Appeal 2007 (RPBA 2007), it is a matter of discretion of the board whether or not evidence filed for the first time in appeal proceedings, but

which could have been presented in the previous proceedings, is admitted and considered.

3.2 Appellant II submitted that document D17 was filed in direct response to the Patentee's assertions that compounds released during pretreatment from lignocellulose biomass would not inhibit *Trichoderma's* growth, a statement made at the oral proceedings, on which the opposition division based its decision.

3.3 Document D17 was filed in an attempt to rebut Appellant I's assertion and to challenge the unfavourable findings of the contested decision on this point (see paragraphs 7.3.3.2 and 7.3.3.3 of the decision under appeal). Since appellant I's allegation of facts was made first during the oral proceedings in opposition, the board considers that document D17 was filed at the earliest possible stage of the proceedings, namely with appellant II's statement of grounds of appeal.

3.4 In these circumstances the board exercised its discretion according to Article 12(4) RPBA and decided to admit document D17 into the appeal proceedings.

Main request - patent as granted
Article 100(c) EPC - added matter

4. In the decision under appeal, the opposition division found that the subject-matter of the claims extended beyond the content of the application as filed. This finding was contested by appellant I as there was a basis for the term "desired protein" in paragraphs [0052], [0057] and [0058] of the patent application.

5. The board considers that the term "desired protein" cannot be derived directly and unambiguously from

paragraphs [0052], [0057] and [0058] of the patent application.

5.1 First, paragraph [0052] of subsection 6.2 of the patent application relates to "enzyme compositions" used for conditioning a lignocellulose biomass or a composition comprising a lignocellulose biomass and extends to functionally equivalent polypeptides corresponding to one or more domains of the enzyme gene product. An enzyme, or a polypeptide, for the conditioning of lignocellulosic biomass is however not the "desired enzyme" as defined throughout the patent application. It is only subsection 6.3 of the patent application which relates to "production microorganisms" which are used to obtain one or more product(s) made by the microorganisms (see page 15, lines 14-18). The "production microorganism" refers to a species of a microorganism which produces a desired product in a microbial process, or that is itself the desired product of a microbial process (see paragraph [0057]). The desired product may be enzymes, organic acids, amino acids, polysaccharides, lipids, nucleotides and vitamins (see paragraph [0058]). There is no explicit basis in the cited paragraphs for a desired product being a protein other than an enzyme.

5.1.1 Although the production of a desired protein may be rendered obvious on the basis of these paragraphs, this is not the relevant question when assessing any amendment for its compliance with Article 123(2) EPC. The term "implicit disclosure" relates only to matter which is not explicitly mentioned, but is a clear and unambiguous consequence of what is explicitly mentioned (Case Law of the boards of appeal of the European Patent Office, Ninth Edition, July 2019, Chapter II E. 1.3.4.a). Since, there is neither an explicit nor an

implicit disclosure of a protein or polypeptide, other than enzymes, produced by the microorganism, this amendment infringes Article 123(2) EPC.

5.1.2 Decisions T 962/98 and T 1906/11 cited by appellant I are not applicable to the present case as they concerned the extraction of characteristics not closely related to the other characteristics of the working example or the general description and were directly and unambiguously applied to a more general context.

5.2 Summarizing the above, it is concluded that there is neither explicit nor implicit disclosure in the application as filed of a method as defined in claim 1. Consequently, Article 100(c) EPC prejudices the maintenance of the patent as granted.

Twelfth auxiliary claim request (ACR12)

6. Claims 1 to 3 of the twelfth auxiliary claim request (ACR12) differ from claims 1 to 3 of the main request in that claims 1 and 2 define the "... a production microorganism that produces a desired [~~protein~~] enzyme, ... that was not contacted with said phenol oxidizing enzyme, ... " and claim 3 defines a "method for producing a desired [~~protein~~] enzyme, ... that produces said desired [~~protein~~] enzyme ... that was not contacted with said phenol oxidizing enzyme, ... " respectively.

Articles 83, 84, 123(2) and Rule 80 EPC

7. Appellant II raised no objections under Rule 80 EPC and Article 84 EPC against the twelfth auxiliary claim request (ACR12) but did so under Articles 123(2) and 83 EPC. After hearing the parties on these issues, the

board concluded that the twelfth auxiliary request did not contravene said provisions. Since this request is however not allowable for lack of novelty (*infra*), the board sees no need for providing its reasons for these conclusions.

Novelty (Article 54 EPC)

8. The board agrees with the finding of item 4.7 of the decision under appeal and the interpretation of claim 1.

9. Claims 1 to 3 are directed at
- a method for conditioning a lignocellulose biomass,
- a method for culturing a production microorganism, and
- a method for producing a desired enzyme,

respectively,
all of them comprising the step of contacting a composition comprising a lignocellulose biomass, wherein said lignocellulose biomass comprises lignocellulose biomass that has not been subjected to acid pretreatment, with a phenol oxidizing enzyme for a period of time sufficient to condition said composition.
The conditioned biomass is functionally characterised by the feature "wherein the rate of growth of said production microorganism cultured in said conditioned composition is increased relative to that of a composition comprising lignocellulose biomass that was not contacted with said phenol oxidizing enzyme".

9.1 The criteria set out in decisions G 2/88 and G 6/88, can only be readily applied to claims directed to the use of a substance for achieving an effect, but cannot be directly transferred to process claims for producing

a product characterised by process steps wherein the intended use of the resulting product is indicated in the claim (see Case Law of the Board of Appeal of the European Patent Office, Ninth Edition, July 2019, Chapter I.C.8.1.3a and b).

9.1.1 Present claim 1, when reformulated, relates to a process for producing a conditioned lignocellulose biomass indicating the intended use as "for use as a feedstock for a production microorganism that produces a desired enzyme". In view of the starting material and the procedural steps defined in the claim, the claimed process can only serve the purpose of producing a conditioned biomass wherein the product is the necessary result of the mentioned method steps.

9.1.2 In order to achieve its purpose, the method of claim 1 defines that a phenol oxidizing enzyme must be contacted with the biomass composition for a period of time sufficient to condition said composition, such that the rate of growth of a production microorganism cultured in the conditioned medium is increased relative to growth in a composition that has not been contacted with said phenol oxidizing enzyme. The claim defines neither the microorganism nor the experimental conditions necessary to establish whether an increased rate of growth, and to which extent, is achieved or not. Thus, the subject-matter cannot be construed to be novel over any prior art process comprising incubation/ conditioning of lignocellulose biomass with an enzyme for any amount of time, as long as the enzyme can act on the substrate. The very vaguely defined functional feature does not help to delimit the claimed method from any prior art method having the same procedural steps.

9.2 Document D16 discloses hydrolysates of sugarcane bagasse pre-treated by steam explosion at 205 and 215 °C, i.e. a non-acid pretreatment, which are then hydrolysed with cellulolytic enzymes. The hydrolysates were detoxified with phenol oxidase laccase or chemically by overliming, before being used as a substrate for fermentation into ethanol. These hydrolysates were fermented with xylose-utilising Saccharomyces cerevisiae laboratory strain TMB 3001 which integrated and expressed heterologous recombinant D-xylose reductase (XR), xylitol dehydrogenase (XDH), and the S. cerevisiae strain ATCC 96581. The enzymatic treatment was shown to have a marked effect upon the removal of phenolic compounds: approximately 80% of the phenols were removed from both hydrolysate H205 and H215 and glucose was readily fermented in the detoxified hydrolysates, whereas some glucose still remained after 12 h in the undetoxified hydrolysates (page 277, col.2, lines 21-24; Fig. 1 and page 278 col. 2, lines 1-4, Fig.4). An almost two-fold increase of the specific productivity of the strain TMB 3001 was also observed in the detoxified hydrolysates compared to the undetoxified hydrolysates (see abstract, Table 3 and Figure 4).

9.3 The opposed patent mentions further that an increase in growth rate of a production microorganism in a process of the invention can be estimated by a number of parameters, such as, but not limited to, nutrient consumption, catabolite accumulation, pH, cell mass, cell number, etc (see patent col.16, lines 35-39, Fig.1 and [0083]).

In consequence, both the increased consumption of glucose or sugars and the increased production of ethanol (i.e. catabolite accumulation) over time

demonstrate an inevitable growth and establish, according to the patent's own parameters to be tested, that the rate of growth of said production microorganism cultured in said conditioned composition is increased relative to that of a composition comprising lignocellulose biomass that was not contacted with said enzyme.

9.4 Thus, the method described in document D16 comprising the same steps as claim 1 deprives the subject-matter of claim 1 of novelty.

Thirteenth auxiliary claim request (ACR13)

The thirteenth auxiliary claim request (ACR13) corresponds to the twelfth auxiliary claim request but with claims 1, 3 and 10 deleted. New claim 1 was derived from granted claim 2 that defines "a method of culturing a production microorganism that produces a desired [~~protein~~] enzyme, ... that was not contacted with said phenol oxidizing enzyme, ... ".

Articles 54, 83 and 123(2) EPC

10. Appellant II maintained all its objections raised against the previous claim requests under Articles 123(2), 83 and 54 EPC. After hearing the parties on these issues, the board concluded that the thirteenth auxiliary request did not contravene said provisions. Since this request is however not allowable for lack of an inventive step (*infra*), the board sees no need to provide its reasons how it came to these conclusions.

Inventive step (Article 56 EPC)

11. It was common ground between the parties that document D5 represents the closest prior art for the subject-matter of claim 1.

11.1 Document D5 discloses the culture of Trichoderma reesei strain Rut C30 in a fed batch cellulase production system with solids and liquid hydrolysate from bagasse pretreated with hot water but not with acid (see abstract; page 922, lines 27-34). The fungal cell was found to be sensitive to inhibitors in the liquid hydrolysate and to some components in the solids (see abstract line 6). Filtration of the liquid hydrolysate reduced the inhibitory effects, while the solid had to be washed heavily to achieve good growth and cellulase production rates (see Fig. 1 to 5). Lignin or other materials in the lignin solids were expected to cause the lag observed for pretreated bagasse (see abstract, page 927, lines 13-15, page 933, lines 3-6;). Document D5 mentions that the remaining growth inhibition of microorganisms cultured on the filtered hydrolysate: "... was much less for cultures grown on filter-sterilized hydrolysate. In fact, these cultures grew vigorously following an initial lag, ... " (see page 926, lines 2-3).

11.2 The difference between the method described in the closest prior art document D5 and the claimed invention lies in the use of a lignocellulosic biomass which has been contacted with a phenol oxidizing enzyme for a period of time sufficient to condition said composition for microbial growth.

11.3 Appellant I submitted that the use of a lignocellulosic biomass conditioned by a phenol oxidizing enzyme for cell growth resulted in dramatic cell growth, as observed by glucose consumption (see Figure 1: 0.5/2

day and 0.5/3 day), and in the disappearance of the lag phase of the cell culture. Therefore, the technical problem should be defined as the provision of an improved method for culturing a production microorganism.

11.4 The board is not convinced by this argument.
First the wording of claim 1 allows any concentration of phenol oxidizing enzyme to be used over any period of time to condition the lignocellulosic biomass.
Second, Figure 1 of the patent shows, but for the use of 0.5 U/ml of laccase activity over 2 or 3 days, no dramatic cell growth in comparison to a cell culture carried out in a non-conditioned lignocellulosic biomass medium.
Third, neither the description nor Figure 1 of the patent suggests that the culture of the cells would be affected by a lag phase. Hence claim 1 comprises not only methods for culturing a production microorganism comprising the step of contacting a composition comprising a lignocellulose biomass to elicit a dramatic increase in cell growth, but also a large number of methods using lignocellulosic biomass pretreated with phenol oxidizing enzyme for a period of time which may be only slightly higher than that observed for unconditioned lignocellulosic biomass.

11.5 Since the patent did not report a lag phase during cell culture neither in pretreated nor untreated lignocellulosic biomass, the board cannot acknowledge this to be a problem. Either the lag effect existed already when using the cultivation conditions of the prior art or it only existed when using the conditions of the examples, although claim 1 covers a large number of other embodiments for which this lag phase has not been shown to be absent or to be a problem. Absent any

data in the patent suggesting that a lag phase in the cell culture was actually a problem, this aspect, which was either not identified, or did not exist, cannot be taken into consideration in the formulation of the technical problem.

11.6 In the board's view, the problem underlying the present invention may be defined as the provision of an alternative method of culturing a microorganism in pretreated lignocellulosic biomass.

11.7 The solution proposed in claim 1 is to use a lignocellulosic biomass conditioned/pretreated with a phenol oxidizing enzyme for a period of time sufficient to condition said composition.

11.8 It remains to be established whether the claimed subject-matter was obvious or not to a person skilled in the art at the relevant date.

11.9 The board shares appellant I's view that document D5 mentions only that lignin or other materials in the lignin solids could cause the lag observed for pretreated bagasse, but more studies were needed to resolve the actual reason for this delay (see abstract, last sentence). There was also no indication in document D5 that phenolic compounds inhibited the cellular growth.

Document D5 discloses that cells grown on combinations of hydrolysate and 10 g/L of sorbitol as the carbon source, in mixtures ranging from 0 to 15% were not significantly inhibited, but for mixtures with 40% hydrolysate, initial growth was delayed by about 22 hours while cultures with 50% hydrolysate did not grow at all (see page 924, last paragraph; Figure 1). Cell

cultures grown on filter-sterilized hydrolysate were less inhibited as filtration apparently removed toxins or those constituents that became toxic in the autoclave, whereas no growth was observed for 80% hydrolysate (see Fig. 2 and 3). Cells grown on pretreated solids resulted in vigorous growth after extensive washing of the pretreated solids, although an initial lag could not be eliminated (see Fig. 4). Thus, the higher growth rate observed both in the liquid hydrolysate and in the heavily washed solids could be due to the removal of cell growth inhibiting components. The addition of lignin to Solka Floc suggested that either lignin or other materials in the lignin solids might cause the lag observed for pretreated bagasse (see abstract, last sentence, Fig. 9). The liquid hydrolysate fraction which is growth inhibitory to organisms could be reduced by filtering (see page 932, lines 14-20). As more work was needed to pinpoint what caused the lag in enzyme production on pretreated solids, it was suggested that inhibitors caused this delay; the challenge was accordingly to develop approaches to either remove the inhibitors or adapt the organism to their effects (see page 933, last paragraph).

11.10 Appellant I maintained that document D8 referred to twelve different detoxification treatments of lignocellulose hydrolysates to improve the growth of *S. cerevisiae* using either laccase or *Trichoderma reesei* (see Table 2). Documents D8 and D5 relied however on different types of pretreated lignocellulosic biomasses and cells which precluded a combination of their teaching. It was specified that the effect of the different detoxification methods of the prior art could not be compared with each other because different hydrolyzates and different microorganisms were used in

the fermentations (see document D8, page 93, first full paragraph).
Even if laccase treatment affected the concentration of phenolic compounds, i.e. a specific group of inhibitors compared to anion exchange at pH 10, the ethanol productivity and biomass yield were still lower than in the reference fermentation (47% and 61% respectively) (see bridging paragraph on pages 101-102). In contrast, the anion exchange at pH 10 removed most of the furan derivatives, probably owing to hydrophobic interactions with the anion-exchange matrix, resulting in higher productivity and biomass yield (see page 102, lines 23-26, page 97, lines 11-12; Table 2, N°12).

11.11 Although the effects of the detoxification methods of the prior art cannot be directly compared with each other, which applies also to the methods of documents D5 and D8, this is, in the board's view, not required, as the objective technical problem underlying the claimed invention is merely to find an alternative method to the one disclosed in document D5.

11.11.1 It is also irrelevant that document D8 uses acid-treated lignocellulosic biomass instead of non-acid-treated lignocellulosic biomass to increase the ethanol yield in S. cerevisiae and its biomass. The detoxification methods on a pretreated lignocellulosic biomass in document D8 are all suitable alternative methods, even if the anion exchange at pH 10 removed most of the furan derivatives and resulted in higher productivity and biomass yield than laccase treatment which affected only the concentration of phenolic compounds (see paragraph bridging pages 101 to 102; page 102, lines 23-26; page 97, lines 11-12; Table 2, N°12). Several phenolic compounds were identified to be responsible for this inhibition, including a group of

phenolic lignin degradation products referred to as Hibbert's ketones. Thus, document D8 establishes that acid-pretreated lignocellulosic biomass detoxified further with either laccase or *Trichoderma reesei* provided the second highest cellular biomass yield and improved its fermentation (see document D8, Table 2, N°13 and N°15 respectively).

Although document D5 mentioned that liquid hydrolysate obtained after hot water treatment was less toxic to the growth of organisms than hydrolysate obtained after dilute acid pretreatment (page 922, middle of the bottom paragraph), it recognized as well that pretreated biomass needed to be detoxified, as the lignin or other materials in the lignin solids present in the liquid hydrolysate, might cause the delays in enzyme production and the lag phase during the growth of the organism observed for pretreated solid bagasse. This could be eliminated for pretreated solids only after extensive washing (see page 927, lines 11-17, abstract, page 933, lines 7-9).

11.12 Starting with the content of document D5, the skilled person was thus motivated to find a way to remove the inhibitors or to adapt the organism to achieve an, even if only slightly, better cell growth.

11.12.1 Faced with the technical problem of finding an alternative to the method of document D5, the skilled person would have turned to document D8, directed at the removal of inhibitory compounds, such as lignin or lignin derivatives, known to be a problem.

11.12.2 Since the hot water pretreated lignocellulosic biomass contains less toxic compounds than dilute acid-pretreated, possibly owing to greater lignin removal,

and the liquid hydrolysate is less toxic to the growth of microorganisms (see document D5, p.922, lines 31-38), the skilled person would want to remove the remaining lignin-derived inhibitory compounds, by any one of the methods disclosed in document D8, to achieve an even more effective treatment than the one of document D5. Since the increased ethanol yield and growth of S. cerevisiae were attributed to the detoxification step of acid-pretreated lignocellulosic biomass because of the removal of inhibitory compounds generated during the acid-pretreatment (see document D8 Table 2), the skilled person would not have been detracted from using a detoxification step disclosed in document D8, effective on acid-pretreated lignocellulosic biomass, to further reduce the inhibitory effects assigned to the lignin derived compounds obtained in lower concentration during non-acid pretreatment of the lignocellulosic biomass.

11.13 Thus, the skilled person starting from the method described in document D5, faced with the technical problem of providing an alternative method, would have tried, with a reasonable expectation of success, to detoxify the non-acid pretreated lignocellulosic biomass by using either laccase or a strain of *Trichoderma reesei*, which provided the second best treatment compared to anion exchange at pH 10, which however led to a loss of fermentable sugars (see document D8, abstract, last sentence, Table 2 Nos. 12, 13 and 15). The skilled person would thus have arrived at the solution of claim 1 without inventive activity.

Fourteenth auxiliary claim request (ACR14)

The fourteenth auxiliary claim request (ACR14) corresponds to the thirteenth auxiliary claim request (ACR13), but claim 1, derived from granted claim 2, defines "a method of culturing a production microorganism that produces a desired [~~protein~~] enzyme, ... that was not contacted with said phenol oxidizing enzyme, ... and wherein said microorganism is a Trichoderma species".

12. Appellant II had no objections under Articles 123(2) and 84 EPC against this claim request and no further submissions to make regarding objections under Articles 83 and 54 EPC against this claim request. The board concluded that the fourteenth request did not contravene these articles. Since this request however lacks an inventive step (*infra*), the board sees no need to provide its reasons how it came to this conclusion.

Inventive step (Article 56 EPC)

13. Document D5 represents the closest prior art for the subject-matter of claim 1.

13.1 The difference between the method claimed and the one described in document D5, relying on strain Rut C30 of *Trichoderma reesei*, lies in that the method claimed uses a composition comprising a lignocellulosic biomass which has been contacted with a phenol oxidizing enzyme for a period of time sufficient to condition said composition for microbial growth.

13.2 Appellant I repeated all its arguments and submitted additionally that document D5 related to a method of fermenting S. cerevisiae under anaerobic conditions to produce ethanol, whereas claim 1 related to a method of increasing the growth rate of a *Trichoderma* species.

Both species differed in their biology. Second, document D5 disclosed that *Trichoderma* Rut C30 did not grow on 80% conditioned hydrolysates but nowhere that phenolic compounds were the inhibitors of *Trichoderma*. Hence, the use of laccase-producing *Trichoderma* strains or laccase itself to achieve a detoxification was more than questionable. It followed that it was not obvious to transfer a phenol oxidizing enzyme pretreatment of a lignocellulosic biomass from the method of document D8 to the method of document D5.

13.2.1 Document D17 illustrated that growth of Trichoderma was only inhibited when artificially high concentrations of selected lignin derived inhibitors, unlike in the conditioned medium of claim 1, were present in the culture medium. It assessed whether the presence in the culture medium of three monomeric model compounds, vanillin, protocatechuic acid and ferulic acid have an effect on the production of cellulases of *T. reesei* (see abstract, page 1498, lines 13-16). The concentrations of lignin and monomer model compounds were identical: 0.01, 0.03, and 0.05% (w/v) (see p. 1497, lines 27-29). These artificially high concentrations of vanillin in document D17 stood however in contrast to the concentration of 0.12 g/L of vanillin (corresponding to 0.012% (w/v)) determined in the dilute acid hydrolysate of spruce and of steam exploded poplar (see document D8, Table 1; document D10, page 568, Table 2: 0.05% in w/w). The fungal biomass was decreased from 80 mg mycelial dry weight to 47 mg/flask when cultured in presence of 0.01% lignin whereas it was only reduced to 73 mg/flask when cultured in presence of 0.01% vanillin (see page 1498, lines 13-16; page 1499, lines 1-5, Fig.1(a) and (b)). In consequence, the artificially high concentrations of lignin derived inhibitors in the medium of document D17

did not allow any conclusion to be drawn for culture media having a much lower natural concentration of these inhibitors.

13.3 Appellant II argued that even if document D5 and documents D8 or D16 related to different purposes and microorganisms, these differences were not decisive, as the skilled person would have turned to documents and solutions of neighbouring technical fields to solve a technical problem, already solved in the more advanced neighbouring technical field, and would have applied such solutions by analogy with a reasonable expectation of success. There was no reason why a different purpose and microorganism should deter the skilled person from consulting these documents and solutions to solve the problem at hand.

Appellant II noted that document D17 identified that vanillin, a lignin monomer model phenolic compound, was capable of inhibiting Trichoderma's biomass and thus its growth rate (see page 1499, lines 1-5).

13.4 The board notes that even at a concentration of vanillin as low as 0.01%, corresponding to its concentration in pretreated lignocellulosic biomass, the biomass of Trichoderma was still reduced even if the reduction was less than in the presence of a higher unnatural vanillin concentration (see document D17, paragraph 13.2.1 above). The board for this reason cannot accept appellant I's argument that the inhibition exerted by vanillin on *Trichoderma* was negligible or non-existant.

Likewise, phenolic compounds, a group of lignin degradation products referred to as Hibbert’s ketones were also identified in document D8 as one of three

major groups of inhibitors, while one of the best detoxification methods applied on lignocellulosic biomass to increase the microorganism's biomass was laccase.

13.5 Since the method of claim 1 neither specifies the concentration of phenol oxidizing enzyme for the conditioning of the lignocellulose biomass nor an incubation time, the dramatic biomass increase observed under some conditions is not taken up to define the objective technical problem to be solved.

13.6 The problem to be solved may be defined as a further method for culturing a production strain of *Trichoderma* that produces a desired enzyme.

13.7 As for the motivation of the skilled person, the board notes that although *Trichoderma* Rut C30 failed to grow on 80% conditioned hydrolysates, it was nevertheless capable of growing in the presence of 60% conditioned hydrolysates or less (see document D5, Fig.3). This renders the introduction of a detoxification step into the method of document D5 more than desirable.

It follows that absent any particular effect assignable to the selection of a *Trichoderma* species as the producing microorganism, the skilled person starting with the teaching of document D5 and faced with the technical problem of finding an alternative method for culturing a production microorganism would have been motivated to combine the method of document D5 with a detoxification step of document D8, preferably using laccase to condition the lignocellulosic biomass. The skilled person would have arrived at the method of claim 1 without inventive activity.

13.8 Thus, the fourteenth auxiliary claim request (ACR14) lacks an inventive step.

Seventeenth auxiliary claim request (ACR17)

14. The seventeenth auxiliary claim request (ACR17) corresponds to the fourteenth auxiliary claim request (ACR14), but claim 1, derived from granted claim 2, defines "a method of culturing a production microorganism that produces a desired [~~protein~~] enzyme, ... that was not contacted with said phenol oxidizing enzyme, ... wherein said microorganism is Trichoderma species and wherein the lignocellulose biomass is corn stover."

15. Appellant II raised only objections under Article 56 EPC.

16. Appellant I argued that the limitation to corn stover further distinguished the method of claim 1 from the closest prior art. Since the results obtained by different pretreatment methods could not be directly transposed (see document D8, page 92 line 35 to page 93 line 12), this prohibition applied by analogy to the attempt to transpose or extrapolate the results obtained by document D8 to the method of document D5.

17. Appellant II maintained that document D5 referred explicitly to cellulosic biomass such as corn stover as low-cost feedstock for biologic production (see page 921, last paragraph). There was no doubt that the pretreated lignocellulosic biomass comprising lignin produced inhibitors, such as phenolic derivatives, resulting in a lower cellular growth rate.

Since the pretreatment by a phenol oxidizing enzyme of corn stover was not associated with any surprising or remarkable results, the technical problem underlying the claimed invention, starting from the method of document D5, could only consist of the provision of an alternative method.

18. The problem to be solved may be defined as a further method for culturing a production *Trichoderma* microorganism that produces a desired enzyme in another lignocellulosic biomass.

19. The board considers that since all the detoxification methods disclosed in document D8 were assayed on the same dilute acid hydrolysate of spruce (lignocellulosic biomass) in combination with the same S. cerevisiae microorganism, each detoxification method and its effect on the inhibitors can be objectively assessed. Thus, based on document D8 the skilled person was capable to readily select which detoxification method was most suited to remove inhibitors present in a pretreated lignocellulosic biomass.

19.1 The board considers that, for the reasons already developed for the previous claim requests, the skilled person faced with the problem of providing an alternative method to the one of document D5 would have tried with a reasonable expectation of success to incorporate a detoxification step of the pretreated lignocellulosic biomass by conditioning the substrate with either a laccase producing *Trichoderma*-strain or a laccase itself as disclosed in document D8. The mere selection of corn stover instead of bagasse as one of the possible and suitable pretreated lignocellulosic biomasses, disclosed as one of the common cellulosic materials, lacks an inventive step.

The board cannot accept appellant I's argument (see item 13.2.1 above) that the skilled person would have considered the inhibition exerted by vanillin on Trichoderma as negligible or non-existant for the same reasons as developed in item 13.4 above with regard to the fourteenth auxiliary claim request 14 (ACR14).

Eighteenth auxiliary request (ACR18)

20. The eighteenth auxiliary claim request (ACR18) corresponds to the seventeenth auxiliary claim request (ACR17), but claim 1, derived from granted claim 2, defines "a method of culturing a production microorganism that produces a desired [~~protein~~] enzyme, ... that was not contacted with said phenol oxidizing enzyme, ... wherein said microorganism is *Trichoderma* species and wherein the lignocellulose biomass is corn stover; wherein the phenol oxidizing enzyme comprises a laccase produced by a first *Trichoderma* species or a *Stachybotrys* species, and said microorganism cultured in said composition is a second *Trichoderma* species."

21. Appellant II only raised objections under Article 56 EPC.

22. Appellant I argued that claim 1 comprised a laccase produced by a first *Trichoderma* species or a *Stachybotrys* species, and that the microorganism cultured in said composition was a second *Trichoderma* species. There was no reason why the skilled person would add laccase to a Trichoderma species already producing laccase, especially in the context of conditioning a corn stover biomass, as required by claim 1.

23. Appellant II argued that it was logical to add to an insufficient amount of phenol oxidizing enzyme more of said enzyme of a microorganism producing it, in order to achieve the desired effect. The fact that the laccase enzyme was produced by a "first" and "second" strain had no effect on the enzyme activity and could not be ascertained. In any case, they were considered equivalent in the patent. This difference amounted therefore to an arbitrary choice from a number of different solutions, each of which would be obvious to the skilled person.

24. In the board's view, no particular technical effect can be attributed to the use of a laccase produced either naturally or recombinantly by a first *Trichoderma* or *Stachybotrys* species compared to one produced otherwise. The patent provides long lists of laccase sources and considers them all to be equally suitable (paragraphs [0039] to [0042]. Since there is no particular effect attributed to the use of a phenol oxidase from a particular strain of filamentous fungus compared to that obtained from another strain of microorganism, the skilled person would have arbitrarily selected one from many possible sources in order to solve the problem posed and would have arrived at the subject-matter of claim 1 without inventive activity.

24.1 Appellant I's assertion that a skilled person would not have considered adding more laccase to a *Trichoderma* species already producing laccase remains unsubstantiated. On the contrary, document D8 illustrates that the detoxification of pretreated lignocellulosic biomass by either laccase or by *Trichoderma* reesei differs in efficiency (see Figure

1). Hence, the skilled person would not have considered vain the addition of laccase compared to the addition of a *Trichoderma* strain producing endogenous laccases for conditioning lignocellulosic biomass.

25. The board considers that adding laccase to a Trichoderma species already producing laccase amounts to an arbitrary selection from among several possible solutions, each of which is obvious to the person skilled in the art. The same holds true if this choice is combined with the additional selection of corn stover as the source of lignocellulose biomass, which is also not associated with a particular technical effect. It follows that the proposed amendments do not render the subject matter of claim 1 inventive.

Nineteenth auxiliary request (ACR19)

26. Claims 1 to 13 of the nineteenth auxiliary request (ACR19) differ from claims 1 to 14 of the main request in that new claims 1 to 3 specify that the lignocellulose is un-pretreated.

Admission into the appeal proceedings

27. ACR19 was first filed with the proprietor's reply to the opponent's statement of grounds of appeal. It is similar to ACR8 that was filed before the opposition division. No arguments concerning ACR8 were presented either in writing or orally before the opposition division with regard to whether it complied with the provisions of the EPC. The opposition division decided that the higher ranked ACR3 complied with the EPC.

27.1 The board notes that ACR19 was submitted without any indication as to why it should be allowable.

27.2 Article 12(3) RPBA 2020 states that:

> "The statement of grounds of appeal and the reply shall contain a party's complete case. Accordingly, they shall set out clearly and concisely the reasons why it is requested that the decision under appeal be reversed, amended or upheld, and should specify expressly all the requests, facts, objections, arguments and evidence relied on…".

27.3 The board has a discretion not to admit any submission by a party that does not meet the requirements of Article 12(3) RPBA 2020 – see Article 12(4) RPBA 2007.

27.4 The board notes that ACR19 is not convergent with any of the requests examined by the opposition division and there is no prior art on file dealing with the unpretreated lignocellulose that forms the subject matter of this request. ACR19 thus represents an entirely new case.

27.5 The board further notes that ACR19 was neither considered at first instance nor subject to substantive submissions by the proprietor on appeal.

27.6 ACR19 is thus not a submission that meets the requirements of Article 12(3) RPBA 2020.

27.7 The board thus exercised its discretion under Article 12(4) RPBA 2007 not to admit ACR19 into the proceedings.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated