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Datasheet for the decision of 19 January 2017

Case Number: T 1872/11 - 3.3.08

Application Number: 03731853.2

Publication Number: 1468093

IPC: C12N15/61, C12N15/54, C12N1/14, C12N1/19, C12P7/10, C12P7/20, C12P7/46, C12P7/54, C12P7/56, C12P13/04, C12P35/00

Language of the proceedings: EN

Title of invention:
FERMENTATION OF PENTOSE SUGARS

Patent Proprietor:
DSM IP Assets B.V.

Opponents:
DSM IP Assets B.V.
Cargill, Incorporated

Headword:
Xylose isomerase rumen fungus Piromyces host yeast cell Saccharomyces ethanol production/DSM

Relevant legal provisions:
EPC Art. 107, 83
EPC R. 22, 85

Keyword:
Admissibility of appeal (yes)
Main and sole request - sufficiency of disclosure (yes)
Remittal to the department of first instance (yes)

Decisions cited:
T 1329/04, T 0146/07, T 0390/07, T 0128/10

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

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Case Number: T 1872/11 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 19 January 2017

Appellant: (Patent Proprietor)	DSM IP Assets B.V. Het Overloon 1 6411 TE Heerlen (NL)
Representative:	Swinkels, B.W. Nederlandsch Octrooibureau P.O. Box 29720 2502 LS The Hague (NL)

Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 28 June 2011 revoking European patent No. 1468093 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	P. Julià
	D. Rogers

Summary of Facts and Submissions

I. Two oppositions were filed against European patent no. 1 468 093 on the grounds as set forth in Article 100(a),(b) and (c) EPC. The opposition division revoked the patent on the grounds that the main request (claims as granted) and auxiliary request 1 contravened Article 123(2) EPC and that auxiliary request 2 did not fulfil the requirements of Article 83 EPC.

II. On 29 July 2011, opponent 01 withdrew its opposition.

III. An appeal was lodged by the patent proprietor (appellant) on 26 August 2011. With the statement setting out the grounds of appeal filed on 8 November 2011, the appellant submitted new documentary evidence (D43-D52) and a main request identical to auxiliary request 2 underlying the decision under appeal. The appellant requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request, or alternatively, that the main request be remitted to the opposition division for further prosecution.

IV. Opponent 02 (respondent) replied to the statement of grounds of appeal and filed new documentary evidence (D53-D74). The respondent requested that the appeal be declared inadmissible or, alternatively, be dismissed or, if it was admitted and not dismissed, be remitted to the opposition division for further prosecution.

V. As an auxiliary measure, oral proceedings were requested by both parties.

VI. Unsigned third party observations were filed. With reference to decision T 146/07 of 13 December 2011, the parties were informed that these observations were deemed not to be filed and excluded from public inspection. Nevertheless, they were erroneously put into the part of the file open to public inspection. The board referred to decision T 390/07 of 20 November 2008 and noted that the admissibility of these observations was a matter to be decided by the board. Three further (signed) third party observations were filed. The appellant provided evidence to cast serious doubts on the identity of two of them and addressed the objections raised in the third.

VII. The parties were summoned to oral proceedings. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), they were informed of the preliminary, non-binding opinion of the board on some issues of the case.

VIII. In reply to the board's communication, the appellant filed further submissions addressing only the admissibility of the appeal.

IX. Under cover of a letter dated 13 December 2016, the respondent (opponent 02) withdrew its opposition.

X. Oral proceedings were held on 19 January 2017 in the presence of the appellant.

XI. The following documents are cited in this decision:

D14: M. Kuyper *et al.*, FEMS Yeast Research, 2003, Vol. 4, pages 69 to 78;

D18: M. Kuyper *et al.*, FEMS Yeast Research, 2005,

Vol. 5, pages 399 to 409;

D32: Experimental evidence from Paul Klaassen, DSM Food Specialties B.V., 17 March 2011;

D33: E.W. Jones in "Methods in Enzymology", 2002, Vol. 351, pages 127 to 150;

D53: Deed of assignment between Royal Nedalco B.V. and C5 Yeast Company B.V., filed on 9 November 2011;

US 5,238,822 (publication date 24 August 1993): document filed as "Third party observations" under cover of a letter dated 25 November 2013.

XII. Claims 1, 7, 10, 12 and 16 of the main request read as follows:

"1. A yeast or filamentous fungal host cell transformed with a nucleic acid construct comprising a nucleotide sequence encoding a xylose isomerase comprising an amino acid sequence that has at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 1, as determined using a Needleman and Wunsch algorithm, the BLOSSUM 62 comparison matrix, a Gap penalty of 12 and a Gap length penalty of 4, whereby the nucleic acid construct, upon transformation of the host cell, confers to the host cell the ability of isomerising xylose to xylulose, which confers to the host cell the ability to grow on xylose as carbon source.

7. A transformed host cell according to any one of the preceding claims, whereby the host cell comprises a genetic modification that results in a characteristic selected from the group consisting of:
(a) increased transport of xylose into the host cell;

(b) increased xylulose kinase activity;
(c) increased flux of the pentose phosphate pathway;
(d) decreased sensitivity to catabolite repression;
(e) increased tolerance to ethanol, osmolarity or organic acids; and
(f) reduced production of by-products.

10. A transformed host cell according to any one of the preceding claims, whereby the host cell expresses one or more enzymes that confer to the host cell the ability to produce lactic acid, acetic acid, succinic acid, amino acids, 1,3-propane-diol, ethylene, glycerol, ß-lactam antibiotics and cephalosporins.

12. A process for producing ethanol, whereby the process comprises the steps of:
(a) fermenting a medium containing a source of xylose with a transformed host cell as defined in any one of claims 1 - 9, whereby the host cell ferments xylose to ethanol, and optionally,
(b) recovery of the ethanol.

16. A process for producing a fermentation product selected from the group consisting of lactic acid, acetic acid, succinic acid, amino acids, 1,3-propane-diol, ethylene, glycerol, ß-lactam antibiotics and cephalosporins, whereby the process comprises the steps of:
(a) fermenting a medium containing a source of xylose with a transformed host cell as defined in claims 10 or 11, whereby the host cell ferments xylose to the fermentation product, and optionally,
(c) recovery of the fermentation product."

Claims 2-6 and 8-9 were directed to specific embodiments of claims 1 and 7, respectively. Claim 11

defined a preferred embodiment of claim 10, and claims 13-15 and 17 were directed to specific embodiments of claims 12 and 16, respectively.

XIII. An important issue in the decision under appeal was whether the yeast strain "BJ1991" was at all suitable for assaying xylose isomerase activities as described in Examples 2 and 3 of the patent. In point 4.3.4 of the decision under appeal, the opposition division stated the following:

"*In D32, yeast strain BJ1991 transformants expressing the Piromyces sp. E2 xylA gene on plasmid pYES2 were inoculated in SC-medium with 100 mM xylose and 25 mM galactose, as described in examples 2 and 3 of the patent. Consumption of the sugars was verified by NMR analysis. The results indicate that xylose consumption did not take place, and that also the amount of galactose consumed was minimal. Closer inspection of the genotype revealed that yeast strain BJ1991 has a gal2 mutation, i.e. a mutation in the gene encoding galactose permease, which is responsible for the uptake of galactose by yeast cells. The genotype of BJ1991 is shown in D33, page 148. As a consequence of the gal2 mutation, the BJ1991 strain cannot take up galactose. This has serious implications since the pYES2 plasmid has a GAL-1 promoter which needs galactose for induction. The Piromyces sp. E2 xylA gene can thus not be expressed from this plasmid in the absence of galactose.*"

In view of the evidence provided in documents D32 and D33, the opposition division considered that there was either an error in the name of the yeast strain BJ1991 or in the genotype indicated in Examples 2 and 3 of the patent. The skilled person trying to reproduce Examples

2 and 3 was however unaware of this problem and would have ordered the strain BJ1991. Only after being unable to obtain the results shown in these examples, a skilled person, as one of many possibilities, would have verified the genotype of the strain. The identification of the gal2 mutation as a cause of the failure was considered not to be straightforward. Rather than verifying the genotype of the strain, a skilled person would have used the second strain (CEN.PK113-5D) exemplified in the patent. However, document D32 reported that the growth profiles shown in Figure 1 of the patent could not be reproduced with this strain when transformed with the xylose isomerase from *Piromyces* sp. E2. As shown in document D14, the plasmid (pYES2XylA) used in Examples 2 and 3 and in document D32 comprised an open reading frame that resulted in a xylose isomerase longer than the original xylose isomerase and with a reduced activity. None of the strains transformed with this plasmid were able to grow on xylose as a carbon source. A strain capable of growing on xylose would have been obtained only by following the teachings of Example 4. In this example, a CEN.PK113-5D strain was transformed with a vector derived from pYES2 in which the GAL-1 promoter was replaced with the TPI1 promoter (constitutive expression) and the original stop codon in the xylose isomerase gene was restored. The skilled person, unaware of the deficiencies identified in documents D32 and D14, needed a good portion of luck when trying to carry out the invention over the entire scope of the claims, since a variation of either the yeast strain, promoter or cloning site could result in a strain unable to grow on xylose as carbon source. The assessment of this functional feature of claim 1 was considered to be highly important by the opposition division.

The opposition division concluded that Figure 3 of document D14 was the sole evidence showing that a strain obtained following the teachings of Example 4 was capable of growing on xylose as carbon source. However, this strain exhibited such a slow growth rate (0.005 h^{-1}) that a skilled person would have doubted whether it actually solved the technical problem defined in the patent ("*the provision of a xylose isomerase that could be expressed in yeast to provide sufficient xylose isomerase activity under physiological conditions to allow for the use of xylose as carbon source*"; page 2, line 58 to page 3, line 2). Therefore, the skilled person would have read the claim in the light of the description of the patent which, as the sole testable parameter, required a growth rate of at least 0.01 h^{-1} on xylose as carbon source (page 6, paragraph [0027]). The growth rate of the strain of Example 4 did not meet this requirement. Although the growth rate could allegedly be increased by changing the medium or manipulating downstream genes, the patent did not disclose any specific conditions under which the growth rate had to be measured. Example 4 did not specify the particular culture conditions and, in the description of the patent, only a general list of possible downstream genetic modifications was given, it represented only an invitation to start a research program. The skilled person, even if reproducing the only exemplified transformed strain capable of growing on xylose, was thus left guessing under which conditions this strain had to be grown in order to arrive at the claimed invention. Given these deficiencies and the lack of information regarding the culture conditions, the opposition division considered that the invention could not be reproduced without undue burden.

XIV. The submissions of the appellant, insofar as they are relevant to the present decision, may be summarized as follows:

Article 83 EPC

The expression of heterologous enzymes in yeast and filamentous fungi was well established and within the common general knowledge in the field at the priority date of the patent. The patent referred to several prior art documents, including textbooks and standard handbooks, concerned with means and methods required for the transformation of such hosts and the expression of heterologous enzymes, such as media and protocols for host cell growth, nucleic acid constructs with preferred promoters, terminator sequences, etc. No undue burden was required for a skilled person to obtain transformed yeast or filamentous fungi expressing high levels of the xylose isomerase from *Piromyces* sp. E2 (SEQ ID NO: 1) disclosed in the patent. Examples 2 and 3 of the patent were not representative for the choices a skilled person would have made in view of this prior art and the information disclosed in the patent. The skilled person would not have destroyed the original stop codon of the open reading frame encoding the xylose isomerase and would not have chosen the GAL-1 promoter used in these examples because, contrary to the promoters described in the patent as preferred promoters, the GAL-1 promoter was sensitive to catabolite repression. The *S. cerevisiae* strain BJ1991 used in Examples 2 and 3 was not disclosed as having a "*gal2*" genotype. US patent No. 5,238,822 showed that, at the priority date of the patent, an *S. cerevisiae* strain BJ1991 with a (gal^+) genotype was available in the prior art. The person

skilled in basic yeast molecular biology would not have chosen a yeast strain deficient in galactose uptake as a host cell for expression from a promoter that required galactose for induction. The results of document D32 were obtained with the wrong *S. cerevisiae* strain BJ1991 and thus, the evidence provided by document D32 was not obtained according to the instructions given in the examples of the patent ("Case Law of the Boards of Appeal of the EPO", 8th edition 2016, II.C.8, page 362).

Example 4 described the construction of an expression vector with a preferred (constitutive, insensitive to catabolite repression) TPI1 promoter that was known, easily available to a skilled person and had been extensively used in the art for expressing heterologous products. Neither the construction of an expression plasmid with this promoter represented an undue burden for a skilled person, nor the transformation of the yeast cells of Example 4 (CEN.PK113) which had no deficiency in sugar transport. The transformants were reported in Example 4 to have a high xylose isomerase activity (two orders of magnitude higher than ever described in the prior art). This level of activity was a remarkable breakthrough in the art making credible that the strains of Example 4 had all the features of claim 1, in particular the ability to grow on xylose as a carbon source conferred by the ability of the cloned enzyme to isomerize xylose to xylulose. The patent disclosed how to measure the growth on xylose as carbon source and the activity of the cloned xylose isomerase by well-known methods already described in the prior art.

The teachings of the patent were not limited to the exemplified construct with the specific promoter and

strain disclosed in Example 4 but provided for alternatives that could have led the skilled person to the desired products ("Case Law", *supra*, II.C.5.1, page 338). The disclosure of the patent, in particular the quality of the evidence in Example 4, went beyond mere speculation and thus, post-published evidence that only confirmed this disclosure could, in accordance with the case law ("Case Law", *supra*, II.C.5.8, page 344, and, *inter alia*, T 1329/04 of 28 June 2005), be taken into account.

Figure 3 of document D14 provided experimental evidence that the strain of Example 4 was capable of growing on a minimal synthetic medium with xylose as sole carbon source, even though at a low rate. However, no particular growth rate was defined in claim 1 which did not even require xylose as the sole carbon source. There was thus also no need for requiring a minimal growth rate of at least 0.01 h^{-1}, let alone on xylose as sole carbon source. Table 1 of document D14 showed that the transformed strain consumed xylose (20-50%) when in (aerobic and anaerobic) chemostat cultures with mixtures of glucose and xylose. Example 4 was a first proof of principle of a pioneering invention that could be further optimized. The higher growth rates referred to in the patent were related to foreseen commercial embodiments that could be obtained by further improvement of the disclosed strains, such as by the genetic modifications described in the patent itself.

Document D14 showed that the ethanol yield was significantly higher in anaerobic xylose-consuming cultures of the transformed strain than in the two reference situations (non-transformed strain grown on mixed substrate and transformed strain grown only on glucose). The volumetric ethanol productivity and the

ethanol yield calculated from Table 1 of document D14 were above the lower limits defined in claims 14 and 15. Evidence on file, such as Table 5 of document D18, showed that yeast strains, falling within the scope of claim 1 and without any further genetic modifications, exhibited high ethanol production and high growth rates (0.01-0.03 h^{-1}) on xylose. This evidence showed that yeast strains genetically modified as described in the patent exhibited even higher growth rates on xylose. For each of the described modifications, the patent referred to prior art publications wherein these modifications had already been successfully carried out. The modified strains were also shown to produce a large variety of fermentation products, including ethanol, glycerol, acetate, succinate and lactate (*inter alia*, Table 5 of document D18). The skilled person was taught by the patent to select host cells with properties suitable for producing the desired fermentation product and both these cells and their properties were well-known in the art and easily available to the skilled person. Thus, on the basis of the disclosure of the patent, the references to the cited prior art and its common general knowledge, a skilled person could have achieved strains with the properties of claims 7 to 11, 14 and 15 without any inventive effort or undue burden. Moreover, apart from claim 1, none of the other claims had been specifically objected to during these proceedings, and no serious doubts had been raised, let alone supported by verifiable facts, about the sufficiency of disclosure as regards these other claims.

Remittal to the department of first instance

For reasons of procedural economy, it made no sense to remit the case to the department of first instance.

Submissions and arguments on Article 56 EPC were already on file and, based on them, there was no reason for the board not to take a decision on the requirements of this article.

XV. The patent proprietor (appellant) requested that the decision under appeal be set aside and that the patent be maintained upon the basis of the claims of the main request filed under cover of a letter dated 8 November 2011.

Reasons for the Decision

Admissibility of the appeal

1. The former respondent-opponent 02 challenged the admissibility of the appeal: first upon the basis that the wrong party was named as the appellant in both the notice and statement of grounds of appeal; and second upon the basis that the current proprietor and appellant is a former opponent and hence cannot be adversely affected by the decision under appeal. These issues were taken up by the board in a communication.

Wrong party as appellant

2. The board notes that the notice and grounds of appeal were filed on time, the grounds of appeal being filed on 8 November 2011, the last day for doing so. At the time of filing of the above, the EPO's records showed that the proprietor of the patent was Royal Nedalco BV.

One day later, on 9 November 2011, an assignment of the patent in suit from Royal Nedalco BV to C5 Yeast

Company BV was filed with the EPO (document D53). On the same day the fee for registering the transfer was paid.

Rules 22 and 85 EPC govern the registration of such transfers – see also Rule 100(1) EPC and T 128/10 of 10 December 2010. These rules also apply during appeals. Rule 22(3) EPC provides that such transfers shall have effect *vis-à-vis* the EPO only at the date when and to the extent that the documents referred to in Rule 22(1) EPC have been produced. These documents were produced on 9 November 2011, hence it is from 9 November 2011 that the EPO is to consider C5 Yeast Company BV as the owner of the patent. Prior to this date the owner was Royal Nedalco BV as far as the EPO was concerned. The board therefore concludes that the appellant was correctly named in both the notice and grounds of appeal (cf. T 128/10, *supra*, point 3 for application of Rule 22 EPC).

Adversely affected

3. The board notes that the EPO's records show that DSM IP Assets BV, the former opponent 01, is now the proprietor of the patent and the appellant in this case.

The former respondent-opponent 02 argued that the appellant is not adversely affected by the opposition division's decision, and hence, under Article 107 EPC is not entitled to appeal as the appellant can be considered to be the opponent, who is clearly not adversely affected by the opposition division's decision.

The board is unable to find any support in the EPC or the case law of the boards for such an interpretation of "adversely affected" under Article 107 EPC. C5 Yeast Company BV, (and its predecessor in title), was the proprietor of the patent in suit, and hence, adversely affected by the opposition division's decision. DSM IP Assets BV, a former opponent, is now the owner of the patent and is also adversely affected by this decision. Such changes of position are an entirely normal and unobjectionable aspect of commercial life.

4. All other formalities having been complied with, the board therefore finds the appeal to be admissible.

Main request
Articles 123(2) and 84 EPC

5. In the decision under appeal, the opposition division acknowledged the subject-matter of the present main request to fulfil the requirements of Articles 123(2) and 84 EPC (cf. pages 7-8, points 4.1-4.2 of the decision under appeal). The board sees no reason to deviate from the findings of the opposition division as regards these articles (cf. points 22-27 of the board's communication pursuant to Article 15(1) RPBA).

Article 83 EPC

6. Claim 1 is directed to "*a yeast or filamentous fungal host cell transformed with a nucleic acid construct comprising a nucleotide sequence encoding a xylose isomerase*" which is defined by a structural feature, namely to have "*at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 1 as determined ...*", and a functional feature, namely that "*... the nucleic*

acid construct, upon transformation of the host cell, confers to the host cell the ability of isomerising xylose to xylulose, which confers to the host cell the ability to grow on xylose as carbon source" (cf. point XII *supra*). This functional feature is not further defined in claim 1 and there is no reason to read additional limitations into this definition, such as a minimal degree of efficiency of the isomerization, even though it must be sufficient to "*confer to the host cell the ability to grow on xylose as carbon source*". Moreover, claim 1 neither defines specific growth conditions nor specific growth media and thus, it does not exclude the presence of carbon sources other than xylose, such as the mixtures of xylose with either galactose or glucose used in Examples 2-4 of the patent.

The disclosure of Examples 2 and 3

7. In Example 2 of the patent, transformed cells of *S. cerevisiae* strain BJ1991 are grown first on a glucose medium and then on a galactose medium so as to induce the expression and production of the xylose isomerase from *Piromyces sp.* E2 with amino acid sequence NO: 1 (cf. page, 10, paragraph [0054]). In Example 3, transformed yeast cells of the same strain are grown in a medium with 100 mM xylose and 25 mM galactose as carbon sources and growth is monitored by measuring the increase in optical density at 600 nm (OD_{600})(cf. page 10, paragraph [0056]). The results of these experiments are reported in paragraph [0057] and shown in Figure 1 of the patent. Whilst the cultures with the non-transformed *S. cerevisiae* cells stop growing at about 80 hours, the transformed cells do not and the OD_{600} further increases up to at least 150 hours, allegedly due to the cells' ability to grow on xylose, i.e. as

required by claim 1 (cf. page 9, point 4.3.2 of the decision under appeal).

8. The pYES2 vector used in these examples contains the yeast GAL-1 promoter which allows inducible expression of the cloned xylose isomerase. In Examples 2 and 3, the transformed yeast host cell is identified as the strain BJ1991 with the genotype "*matα, leu2, trp1, ura3-251, prb1-1122 and pep4-3*" (cf. page 10, lines 23-24 and 50).

9. Based on the experimental evidence described in document D32, the opposition division concluded that Examples 2 and 3 could not be repeated without undue burden (cf. point XIII above, and page 11, point 4.3.12 of the decision under appeal).

10. For the experiments shown in document D32, a strain of *S. cerevisiae* designated BJ1991 was obtained from the "*American Type Culture Collection (ATCC)*". The Table on page 148 of document D33 shows that the strain BJ1991 deposited with the ATCC under number 208275 has not only the genotype indicated in the patent but also a "*gal2*" genotype, i.e. a deficiency in galactose uptake. The skilled person knows that the induction of the GAL-1 promoter with galactose in the ATCC strain deficient in galactose uptake does not lead to the expression of the cloned xylose isomerase gene, and the experimental evidence provided in document D32 confirms this. Contrary to Figure 1 of the patent, the non-transformed control cells and the transformed cells of the ATCC strain BJ1991 show the same growth profile (cf. Figures 3a) to 3c) of document D32). On the basis of these results, the opposition division concluded that the description of the strain BJ1991 in Examples 2

and 3 was erroneous and that the Examples were therefore not reproducible without undue burden.

11. As pointed out by the appellant at the oral proceedings before the board, there is however evidence on file that strains of *S. cerevisiae* BJ1991 with a "*GAL+*" genotype, i.e. strains with the ability to take galactose up, were also available (cf. US patent number 5,238,822, column 8, lines 54-56 and column 9, lines 10-13 and 20-23, referring to strain BJ1991 as "*GAL+*"; this strain was deposited at the "*National Collection of Industrial and Marine Bacteria (NCIMB)*" under the accession number NCIMB 40243).

12. The purpose of Examples 2 and 3 of the patent is the expression and production of the xylose isomerase from *Piromyces* sp. E2. The yeast GAL1-promoter in the pYES2 vector is used for the inducible expression of this xylose isomerase. Therefore, the skilled person would not choose as the host cell a yeast cell with a deficiency in galactose uptake. A skilled person would certainly have considered the genotype of the BJ1991 strain to be highly relevant and, when reproducing the experiments described in Examples 2 and 3 of the patent, would have used a BJ1991 strain with the genotype described in the Examples, such as the strain deposited as NCIMB 40243. No undue burden would be required for the skilled person to select such a host cell in order to reproduce the teachings of Examples 2 and 3.

13. Document D32 (cf. pages 11-17, Experiment 2) provides further experimental evidence that host cells with the ability to take galactose up (strain CEN.PK113-5D used in Example 4), transformed with the nucleic acid construct of Examples 2 and 3 and grown under the

culture conditions disclosed in these Examples (100 mM xylose and 25 mM galactose), have the same growth profile as non-transformed *S. cerevisiae* cells. Several reasons have been given to explain this result, namely the absence of the original stop codon in the open reading frame encoding the xylose isomerase and the presence of a longer C-terminus (7 residues) which results in a significant decrease of xylose isomerase activity (cf. page 73, point 3.3 of document D14; Example 4 of the patent), an insufficient amount of galactose for the induction of the GAL1-promoter, etc. (cf. page 17, bottom paragraph of document D32). The board has no doubts that a skilled person would have identified these deficiencies and overcome them in a straightforward manner, in particular, in view of the specific instructions in Example 4 to restore the original stop codon and the statement on page 6 (paragraph [0031], lines 45-46) of the patent that a preferred promoter is insensitive to catabolite (glucose) repression.

14. The evidence provided in document D32 is therefore insufficient to cast serious doubts on the reproducibility of Examples 2 and 3.

Reproducibility of Example 4

15. In Example 4, the GAL1 promoter in the pYES2 vector is replaced by a TPI1 promoter so as to ensure a constitutive expression of the xylose isomerase. The open reading frame of the xylose isomerase with the restored original stop codon is cloned in this vector and the resulting plasmid is used to transform the *S. cerevisiae* strain CEN.PK113-5D (genotype *MatA ura3-52*). The transformants are grown in carbon-limited chemostat cultures with a glucose/xylose mixture and reported to

exhibit "*high xylose isomerase activities (800 units per mg at 30°C)*" (cf. page 11, paragraph [0058] of the patent). There is however no information on the specific culture conditions used and there are no data to demonstrate that the "*ability of isomerising xylose to xylulose ... confers to the host cell the ability to grow on xylose as carbon source*" (*supra*).

16. As stated in point 6 above, claim 1 does not require any particular level of activity for the cloned xylose isomerase nor is the claim limited to any specific host strain, promoter, culture conditions or growth rate on a particular sugar or sugar mixture. Example 4 of the patent provides a method for selecting transformed host cells and a method for measuring the specific activity of the xylose isomerase (cf. page 11, lines 25-31 of the patent). Growth media are disclosed in paragraph [0056] of Example 3 and in the general part of the description (cf. page 8, paragraphs [0039] to [0041], page 10, paragraph [0056] of the patent). In the light of this disclosure, the skilled person is in a position to readily and without undue burden establish appropriate culture conditions for growth of a transformed yeast cell on xylose as a carbon source.

17. This conclusion is supported by the post-published document D14.

18. Document D14 describes the transformation of *S. cerevisiae* strain CEN.PK113-5D with vectors pAKX001 and pAKX002 derived from plasmids pYES2 and pPICZα. In both vectors, the GAL1 promoter is replaced by the TPI1 promoter but the original stop codon in the open reading frame of the xylose isomerase is restored only in the pAKX002 vector. The isomerase encoded by the pAKX001 vector is thus seven amino acids longer. Whilst

a low xylose isomerase activity is detected for the longer enzyme (0.025 U/mg), a much higher activity is detected in extracts of host cells transformed with pAKX002 (1.1 U/mg) (cf. page 71, paragraph bridging left and right-hand columns, page 73, point 3.3). In extracts of chemostat cultured cells, the isomerase activity ranges from 0.32 to 1.1 U/mg depending on the (anaerobic/aerobic) culture conditions (cf. page 74, left-hand column, second paragraph). Figure 3 shows that, contrary to the non-transformed strain CEN.PK113-7D, *S. cerevisae* strain CEN.PK113-5D transformed with pAKX002 (*S. cerevisae* strain RWB 202) grows in shake-flasks cultures on synthetic medium with 2% (w/v) xylose as the sole carbon source, albeit at a very slow specific growth rate (0.005 h^{-1}) (cf. page 74, Figure 3, page 75, point 4.3). Whilst in chemostat cultures fed with mixtures of glucose and xylose (2:1) xylose consumption is negligible in the reference strain, "*the strain expressing the Piromyces xylose isomerase consumed 20-50% of the xylose in the feed in aerobic as well as anaerobic cultures*" (cf. page 73, Table 1 and page 74, paragraph bridging left and right-hand columns). Thus, as acknowledged by the opposition division (cf. page 13, point 4.3.16 of the decision under appeal), post-published document D14 provides evidence that, following the teachings of Example 4 of the patent, the skilled person arrives at a host cell having the structural and functional features of claim 1.

19. Moreover, the disclosure of the patent as a whole is not limited to the specific constructs exemplified in the patent but provides for possible alternatives, such as promoters and transcription termination sequences (cf. page 6, paragraphs [0031] and [0032] of the patent), and it also contemplates further specific

genetic modifications (cf. page 6, paragraph [0028]) which correspond to the subject-matter of *inter alia* claims 7 and 8. These modifications are well-known to a person skilled in the particular technical field of the patent because, as acknowledged in the post-published document D14, they are directly derivable from "*many of the concepts developed in the extensive, painstaking research on S. cerevisiae strains expressing heterologous xylose reductase and xylitol dehydrogenase*" (cf. page 76, left-hand column, second paragraph of document D14). This general prior art is also referred to and explicitly acknowledged as the "*Background of the invention*" in the patent itself (cf. page 2, paragraphs [0004] to [0006] of the patent).

20. Post-published document D18 describes *S. cerevisiae* strains derivable from the strain "RWB 202" disclosed in document D14 and produced according to Example 4 of the patent. In particular, this document describes the *S. cerevisiae* strain "RWB 202-AFX" (anaerobic fermentation xylose) obtained by evolutionary engineering and the *S. cerevisiae* strain "RWB 217" comprising some of the genetic modifications referred to in the patent and contemplated in claims 7-9. These strains have a high specific growth rate in both xylose and glucose-xylose mixtures when compared to the reference *S. cerevisiae* strain CEN.PK113-7D (cf. page 405, Table 5 of document D18).

21. Both post-published documents D14 and D18 acknowledge that the *S. cerevisiae* strain "RWB 202" transformed with the xylose isomerase from *Piromyces* sp. E2 is capable of producing ethanol by anaerobic xylose fermentation but not at rate sufficiently high for industrial application (cf. page 400, left-hand column, third paragraph of document D18). In Table 1 of

document D14, the fluxes of (glucose/xylose) sugars and ethanol as well as the biomass and ethanol yield and xylose isomerase activity of (aerobic and anaerobic) chemostat cultures are indicated for both, strain "RWB 202" and the reference strain CEN.PK113-7D. Table 1 shows the production of ethanol by strain "RWB 202" and, according to appellant's calculation presented at the oral proceedings, at a quantitative level as defined in claims 14 and 15 (ethanol per litre per hour and ethanol per gram glucose or xylose).

22. The board observes that the quantitative features of these claims, as well as the features of claims 7-11 and 16-17 concerned with further genetic modifications and the production of fermentation products other than ethanol, were only addressed in a very general manner by the opposition division in the decision under appeal. No comments were made by the then respondent (opponent 02) in reply to the appellant's statement of grounds of appeal and to the board's communication pursuant to Article 15(1) RPBA as regards these claims. As stated above, the post-published evidence on file shows that no particular technical problems or difficulties were encountered when carrying out these modifications in *S. cerevisiae* strains transformed with the xylose isomerase from *Piromyces* sp. E2 disclosed in the patent. Therefore, this post-published evidence, in particular document D14, merely confirms the teachings of the patent and does not remedy any insufficiency of disclosure (cf. "Case Law", *supra*, II.C.5.8, page 344).

23. Thus, the main request fulfills the requirements of Article 83 EPC.

Remittal to the first instance for further prosecution

24. In the decision under appeal, the opposition division considered only the requirements of Articles 123(2), 84 and 83 EPC. No decision was taken as regards the compliance with Articles 54 and 56 EPC. Although submissions were made by the parties on Article 56 EPC, at the beginning of the appeal proceedings, both parties, the appellant and the then respondent (opponent 02), requested, as an auxiliary measure, to remit the case to the department of first instance for further examination if the board decided in appellant's favour on Article 83 EPC (cf. points III and IV *supra*). Submissions from a third party as regards Article 56 EPC were also on file (cf. point VI *supra*). In view of these requests, the board made some general observations regarding Article 56 EPC in its communication pursuant to Article 15(1) RPBA but it did not enter into a detailed analysis of the parties' submissions on this issue. Indeed, such analysis was not even performed by the opposition division which, in its communication issued on 21 December 2010 summoning the parties to oral proceedings, acknowledged only the closest prior art document identified by the then parties in the proceedings.

25. Under these circumstances, the board decides to remit the case to the department of first instance for further prosecution.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance for further prosecution upon the basis of claims 1 - 17 of the main request, filed under cover of a letter dated 8 November 2011.

The Registrar: The Chairman:

A. Wolinski B. Stolz

Decision electronically authenticated