

THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

The opinion in support of the decision being entered today (1) was not written for publication in a law journal and (2) is not binding precedent of the Board.

Paper No. 11

UNITED STATES PATENT AND TRADEMARK OFFICE

Entered
January 7, 1998
BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte ROLAND KURTH

Appeal No. 93-4005
Application 07/795,158¹

ON BRIEF

Before: STONER, Chief Administrative Patent Judge,
and McKELVEY, Senior Administrative Patent Judge,
and ELLIS, Administrative Patent Judge.

McKELVEY, Senior Administrative Patent Judge.

Decision on appeal under 35 U.S.C. § 134

¹ Application for patent filed November 20, 1991. Applicants claim priority under 35 U.S.C. § 119, based on German patent application P 40 37 441.6, filed November 24, 1990.

Appeal No. 93-4005
Application 07/795,158

This appeal is from a decision of the Primary Examiner rejecting claims 3, 4 and 6 as being unpatentable under 35 U.S.C. § 103 over the prior art.

Initially, we note that the examiner's answer (Paper No. 10) creates some confusion as to the claims on appeal. At the time of the final rejection (Paper No. 5), claims 1-6 were in the application. Claims 1-2 were withdrawn from consideration. Claims 3-6 were rejected. An amendment (Paper No. 6) after final cancelled claim 5. In his appeal brief (Paper No. 9), applicant indicates that claims 3, 4 and 6 are on appeal. In the examiner's answer, it is stated that claims 1-5 are rejected (page 2, last two lines) and that patentability stands or falls with claim 6 (page 2, second full paragraph). We agree with applicant that claims 3, 4 and 6 are the claims on appeal.

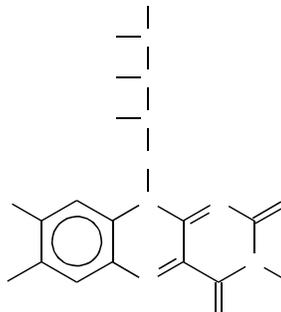
We reverse the examiner's rejection of claims 3, 4 and 6 and enter a new ground of rejection pursuant to 37 CFR § 1.196(b).

A. Findings of fact

Applicant's invention

1. Riboflavin (Vitamin B₂) has the formula:

Appeal No. 93-4005
Application 07/795,158



2. For therapeutic use, riboflavin is produced by chemical synthesis. Kirk-Othmer, Encyclopedia of Chemical Technology, Vol. 24, page 108 and 111-113 (1984).

3. Riboflavin also can be prepared by microbial fermentation of nutrient mashes with the microorganism Ashbya gossypii (Tanner, U.S. Patent N° 2,445,128, col. 1, lines 8-10 (1948); Kirk-Othmer, pages 113-115).

4. Riboflavin prepared for use in poultry and livestock feeds, for example, is produced by fermentation using microorganisms, such as Ashbya gossypii (Kirk-Othmer at 108 and 114; specification, page 1, lines 21-22).

5. Applicant's invention relates to increasing the riboflavin content in spray-dried discharges from riboflavin

Appeal No. 93-4005
Application 07/795,158

fermentations (specification, page 1, lines 3-6). In particular, the invention relates to inducing lysis (i.e., breaking down or dissolution) of the cells contained in the fermentation solution by specific production or activation of the enzymes which break down the intracellular cell wall and proteins, i.e., inducing an autolysis (specification, page 2, lines 25-32).

6. By "autolysis," applicant means "the production and activation of the intrinsic enzymes which break down the intracellular cell wall and proteins" (specification, page 2, lines 33-35).

7. According to applicant, "autolysis of microorganisms can be induced by stress factors" (specification, page 3, lines 3-4). Examples of stress factors are said to include nutrient deficiencies, temperature increases, oxygen deficiencies, pH changes, and changes in the pressure in the medium (specification, page 3, lines 5-8).

8. Applicant tells us that riboflavin-producing strains of Ashbya gossypii can be autolyzed "at from 35 to 55°C within from 6 to 28 hours with almost complete exclusion

Appeal No. 93-4005
Application 07/795,158

of oxygen ***. The pH *** should be from 4 to 9"
(specification, page 3, lines 21-26).

9. Claim 6, the only independent claim on appeal,
reads (indentation ours):

A process for increasing the riboflavin content
in spray-dried discharges from riboflavin
fermentations, which comprises

heating a fermentation solution of riboflavin-
producing strains of Ashbya gossypii after
completing (sic--completion) of the riboflavin
fermentation,

at a temperature of from 40 to 50EC and
a pH of from 4 to 9,
for from 6 to 28 hours,
under gentle stirring without aeration,
to induce lysis of cells by specific production
or activation of the intracellular enzymes,
until complete enzymatic breakdown of the cell wall
and proteins occurs.

Prior art

Appeal No. 93-4005
Application 07/795,158

10. It is known in the art that it is desirable to effect lysis of cell walls in fermentation solutions of Ashbya gossypii to make riboflavin. According to applicant, German Patent 29 20 592² describes (specification, page 1, line 31 through page 2, line 4):

a process for removing riboflavin from fermenter suspensions in which the latter are diluted with from 25 to 100% by volume of water and subsequently heated at from 50 to 65°C for from 15 to 45 minutes in order to open up the cells containing the riboflavin. The suspension is cooled and then centrifuged twice to concentrate riboflavin. In order to increase the riboflavin content, it is recommended that a proteolytic enzyme is added to the diluted broth during the heating or, preferably, thereafter and allowed to act for from 3 to 4 hours.

11. Applicant tells us that there are disadvantages associated with the process described in the German Patent

² German Patent 29 20 592 is similar to Epstein, U.S. Patent N° 4,165,250 cited by the examiner. A translation of the German Patent made by the Patent and Trademark Office accompanies this opinion. One difference is that the German Patent specifically mentions Ashbya gossypii, whereas Epstein does not appear to specifically mention Ashbya gossypii.

Appeal No. 93-4005
Application 07/795,158

29 20 592, including large fermenter suspension resulting from dilution and the cost of proteolytic enzymes (specification, page 2, lines 8-14).

12. The application which matured into the German Patent 29 20 592 was first published on December 4, 1980.

13. According to the German Patent 29 20 592 (page 4):

The starting material used in this invention is a riboflavin fermentation broth. The production of riboflavin from a fermentation broth is known. In brief, with this method a nutrient medium is sterilized and inoculated with a microorganism capable of forming riboflavin, such as Eremothecium ashbyii or Ashbya gossypii. When the fermentation yield reaches its maximum value or approaches this value, then the broth is heated for 15 to 45 minutes (preferably 25 to 35 minutes) at 50 to 65°C, after which the riboflavin extraction begins. The heating causes cell lysis and reduces the viscosity of the broth, which increases the effectiveness of the subsequent extraction and purification stages. Heating for more than 45 minutes is disadvantageous,

Appeal No. 93-4005
Application 07/795,158

since it increases the viscosity of the broth rather than decreasing it.

14. There are four examples in the German Patent 29 20 592. None describe the use of the microorganism Ashbya gossypii. The same is true of the five examples of the Epstein patent.

15. Epstein, like the German Patent 29 20 592, states (col. 1, line 54 through col. 2, line 2):

The starting material for the present invention is a riboflavin fermentation broth. The preparation of riboflavin from a fermentation broth is known ***. Briefly, a nutrient medium is sterilized and inoculated with an organism capable of producing riboflavin. When the fermentation yield approaches or is at about the maximum the broth is heated to a temperature of from about 50EC. to about 65EC. for about from 15 to about 45 minutes, preferably for from about 25 to about 35 minutes and the riboflavin recovery begins. This heating serves to lyse the cells and to decrease broth viscosity thus enhancing the effectiveness of subsequent recovery and purification steps. Heating beyond about 45 minutes is

undesirable as it increases rather than decreases broth viscosity.

16. While both the German Patent 29 20 592 and Epstein make a statement that it is disadvantageous to heat beyond 45 minutes, neither indicate the basis on which this "disadvantage" is based. It is true that both make a statement that viscosity will increase if heating takes place for more than 45 minutes. According to Epstein, "heating serves to lyse the cells" (col. 1, line 66). However, there does not appear to be any experimental data in Epstein to support the conclusion Epstein seeks to draw from heating. There is no basis in Epstein for concluding that the only microorganism mentioned therein (B. subtilis (see, e.g., Example 4)) or Ashbya gossypii would lyse (i.e., would "complete[ly] *** breakdown of the cell wall and proteins," as required by claim 6) in 15-45 minutes at the temperature (50-60°C) described by Epstein. Because the basis upon which the "heating serves to lyse the cells" statement is made is not clear, and in view of later published prior art discussed infra, we conclude that one having ordinary skill in the art would decline to give the statement much weight.

Appeal No. 93-4005
Application 07/795,158

17. After publication of the application which matured into the German Patent 29 20 592 and after the filing date of the application which matured into the Epstein patent, an article was published describing autolysis in yeasts. Babayan, "Autolysis in Yeasts," Acta Biotechnol., Vol. 5, pages 129-136 (1985).

18. According to Babayan, "so-called 'induced autolysis' of microorganism cells of any age, may be artificially caused by means of various physical, chemical, or biological agents" (Babayan, page 130).

19. One physical factor is temperature conditions. According to Babayan, "[t]he autolysis rate depends on temperature, and the most optimal temperature interval for yeast was observed at 45|60°C" (Babayan, page 130, last sentence).

20. One chemical factor is pH (Babayan, page 131).

21. One biological factor is the "[e]xtent of aeration. Removal of oxygen from an actively growing culture of aerobic microorganisms causes impaired energy supply to the cells" (Babayan, page 131).

22. With respect to autolysis, Babayan indicates the following (page 132--emphasis added except for names of microorganisms; references to publications deleted):

In the process of autolysis the cell wall in most yeasts undergoes only certain structural modifications; however, its completeness is retained, this being observed also at the end of the process, and the cytoplasmatic material gradually diffuses into the extra-cellular space. This kind of autolysis ("endo-type") in yeasts is similar to that in Bac. subtilis described by Koga and Kusaka, who observed hollow cells (shadows). In both cases, autolysis begins with CPM [i.e., cytoplasmatic membrane,] degradation.

However, autolysis in certain fungi, e.g. in Schizophyllum commune, is found to involve both CPM and the cell wall degradation, explained by the highly active endo- and exo-glucanases in these organisms.

Autolysis depends on the culture age and the physiological conditions in the cell. Proliferating culture cells autolyse quicker and to a high degree than

Appeal No. 93-4005
Application 07/795,158

cells of the stationary growth phase, for autolytic enzymes are synthesizing most intensively precisely in the cells of the exponential growth phase. Hence, autolysis occurs when induction by means of a given factor not only fails to lead to degradation of autolytic enzymes, but when there are optimal conditions for their action.

What kind of fungi is Ashbya gossypii?

23. In this case, determination of the nature of Ashbya gossypii is important, if not absolutely crucial.

24. If one having ordinary skill in the art would have characterized Ashbya gossypii as a yeast, then the teachings of Babayan would not have been considered particularly relevant to any effort to completely autolyze cell walls, inasmuch as Babayan indicates that yeast cells walls are not completely lysed.

25. On the other hand, if Ashbya gossypii would have been characterized as a filamentous fungi, the teachings of Babayan become relevant, because Babayan teaches that complete autolyzing of cell walls is possible in certain fungi.

Babayan, page 132.

Appeal No. 93-4005
Application 07/795,158

26. According to Davis et al., Microbiology, page 966 (1973) (published by Harper & Row, Publishers, Inc.), "[f]ungi grow either as single cells, the **yeasts**, or as multicellular filamentous colonies, the **molds** and **mushrooms**.

27. In a patent published in 1948, Tanner makes a reference to "the yeast, Ashbya gossypii." Tanner, supra, at col. 1, line 10.

28. By 1961, Smith et al. refer to A. gossypii" as a "mold." See Smith et al., "Effect of Surface Active Agents on the Biosynthesis of Riboflavin by Ashbya gossypii," Biochim. Biophys. Acta, Vol. 47, pages 344-349 (1961), in particular page 344 (emphasis added; footnotes omitted):

A synthetic medium which supports the growth of A. gossypii has been known for some time, but the mold does not produce appreciable quantities of riboflavin except in the presence of complex nutrients such as peptone, corn steepwater, etc.

29. Smith's characterization of Ashbya gossypii as a filamentous mold fungi in 1961 is consistent with the manner in which Ashbya gossypii was characterized in 1991 by the American Type Culture Collection (ATCC) in its Catalogue of

Appeal No. 93-4005
Application 07/795,158

Filamentous Fungi, page 39 (18th ed. 1991). ATCC also publishes a separate catalogue of yeast strains. ATCC maintained its classification of Ashbya gossypii as a filamentous fungi in its 1996 edition: ATCC Filamentous Fungi, page 49 (19th ed. 1996). Likewise maintaining Ashbya gossypii in the category of a filamentous fungi is the German Depository: DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. See copies of materials obtained as a result of an internet search at:
<http://www.gbf-braunschweig.de/DSMZ/media/strains/no003485.htm>

and

<http://www.gbf-braunschweig.de/DSMZ/media/strains/no003499.htm>,

These materials show that Ashbya gossypii samples DSM No. 3485 and DSM No. 3499 for Ashbya gossypii are classified as filamentous fungi.

The examiner rejection and applicant's response

30. The examiner rejected claims 3, 4 and 6 as being unpatentable under 35 U.S.C. § 103 over the combined teachings of Epstein, U.S. Patent N° 4,165,250, issued August 21, 1979 and Babayan.

31. The examiner found that Epstein teaches enhanced recovery of riboflavin by degradation of the undesired cellular macromolecules with enzymes. Examiner's Answer (Paper No. 10, page 3). The examiner recognized that Epstein "does not teach utilizing the cells' own enzymes but adds enzymes to the suspension." Id. Babayan was used to teach that under appropriate conditions, i.e., pH and temperature "yeast cell autolysis" can be induced. Id. According to the examiner, it would have been obvious to use the teachings of Babayan to achieve autolysis, thereby making it unnecessary to add enzymes in accordance with the process suggested by Epstein.

32. The examiner refers to "yeast" cells several times in Examiner's Answer. See pages 3 and 4.

33. Applicant also characterizes Ashbya gossypii as a "yeast" on several pages of the BRIEF ON APPEAL (Paper No. 9), e.g., "the yeast Ashbya gossypii" (page 1); "[a]ccording to the present invention, the riboflavin is released from the yeast cells following fermentation" (page 2).

34. Both the examiner and applicant have based their respective arguments for unpatentability and patentability over

the combination of Epstein and Babayan on an incorrect characterization of Ashbya gossypii as a yeast, when in fact it is a filamentous fungi.

Level of skill in the art

35. In addition to the information revealed in the prior art documents discussed above, one having ordinary skill in the art would have knowledge of the following.

36. One having ordinary skill in the art, as of applicant's filing date, would consider Ashbya gossypii to be a filamentous fungi, not a yeast.

37. Reyes et al. report that "[i]n the filamentous fungi the lytic enzymes involved in the degradation of the cell wall and storage polysaccharides show increased activity in the culture fluid during the autolytic phase of growth. Reyes, "Lytic enzymes in the autolysis of Schizophyllum commune with special reference to 1,3-"-glucanase," Can. J. Microbiol., Vol. 26, page 1120 (1980).

38. Reyes goes on to state that "[t]hese enzymes also occur in the mycelium and in the cell wall" (id.).

39. Reyes indicates that after autolysis has proceeded for some time, "carbohydrates may accumulate and

Appeal No. 93-4005
Application 07/795,158

could cause catabolic repression of enzyme synthesis" (id.) at 1124, end of col. 2. In other words, the optimum time for permitting autolysis to occur must be determined.

40. A first publication of Lahoz et al. reveals that "[i]t is well known that the autolytic phase of growth in filamentous fungi is a degradation [sic-degradation] process carried out to a great extent by lytic enzymes produced by the same fungi." Lahoz et al., "Lytic Enzymes in the Autolysis of Filamentous Fungi," 60 Mycopathologia 45 (1976).

41. Another publication of Lahoz et al. reveals that autolysis of filamentous fungi is influenced by external variables. Lahoz et al., "Effect of the pH on the degree of autolysis of Aspergillus niger," 67 Can. J. Bot. 1901 (1979). Among those external influences are (1) the nature of the nitrogen source, (2) culture conditions, (3) addition of antibiotics or vitamins, (4) temperature, (5) carbon source concentration and, as the article describes, the pH value during autolysis. Id.

B. Discussion

1. The examiner's rejection

Appeal No. 93-4005
Application 07/795,158

As noted above, the examiner found that Ashbya gossypii is a yeast. Applicant seems to agree. But, the evidence we have uncovered reveals that Ashbya gossypii is a filamentous fungi.

The nature of Ashbya gossypii is critical to the evaluation of the prior art cited by the examiner, particularly Babayan. According to Babayan, autolysis of yeasts is difficult. Specifically, Babayan indicates that "[i]n the process of autolysis the cell wall in most yeasts undergoes only certain structural modifications; however, its completeness is retained ***." Babayan, page 132.

Applicant's claim 6, however, requires "complete enzymatic breakdown of the cell wall and proteins ***." Accordingly, if Ashbya gossypii was a yeast, it would be difficult to see how the teachings of Babayan could apply to the claims before us.

The examiner's finding that Ashbya gossypii is a yeast is clearly erroneous. Likewise erroneous, is applicant's statements that Ashbya gossypii is a yeast. Applicant's traverse of the examiner's rejection, like the rejection itself, is bottomed on a faulty factual premise.

Accordingly, we reverse the examiner's rejection.

Appeal No. 93-4005
Application 07/795,158

2. New ground of rejection under 37 CFR § 1.196(b)

Claims 3, 4 and 6 are rejected under 35 U.S.C. § 103 as being unpatentable over (1) Tanner, (2) Kirk-Othmer, (3) German Patent 29 20 592, (4) Babayan, (5) Reyes and (6) the two Lahoz publications.

Tanner and Kirk-Othmer confirm what applicant readily concedes, i.e., "[t]he preparation of riboflavin (vitamin B₂) by microbial fermentation process is known" (specification, page 1, lines 7-8). One microbial fermentation uses the microorganism Ashbya gossypii.

German Patent 29 20 592 reveals that it is desirable to achieve cell lysis (page 4) in making riboflavin a microbial fermentation using Ashbya gossypii as the microorganism.

Babayan teach that in certain fungi (i.e., those which are not yeasts), autolysis involving both cytoplasmatic membrane and cell wall destruction can be achieved through the use of various physical, chemical and biological factors. The second Lahoz article confirms to some extent what Babayan is saying (page 1901, col. 1). The first Lahoz publication tells us that, in the case of filamentous fungi, autolysis is

Appeal No. 93-4005
Application 07/795,158

carried out "to a great extent by lytic enzymes produced by the same fungi" (page 45, col. 1).

Applicant's claim 6 call for the use of certain ranges of temperature, pH and time, as well absence of aeration. All would seem to be result oriented variables. That is, one having ordinary skill in the art, aware of all the prior art cited above, would reasonably expect that autolysis would occur in a fermentation broth containing Ashbya gossypii. However, one having ordinary skill in the art would have had to conduct certain experiments to determine how to balance temperature, pH and time. One biological factor mentioned by Babayan is removal of oxygen. Babayan explains that removing oxygen from actively growing cultures of aerobic microorganisms (Ashbya gossypii is an aerobic microorganism) causes impaired energy supply to the cells (page 131, under Biological Inductors (1)), which one having ordinary skilled in the art would understand would lead to cell death and autolysis. Based on the record before us, including the prior art cited above, we conclude that one having ordinary skill in the art would know what physical, chemical and biological factors are relevant. One having ordinary skill in the art,

Appeal No. 93-4005
Application 07/795,158

based particularly on the teachings of Babayan, would have been able through routine experimentation to determine appropriate ranges for those factors. The prior art, as a whole, demonstrates that one having ordinary skill in the art would have the skill and motivation to determine the optimum conditions to achieve autolysis. Compare In re Woodruff, 919 F.2d 1575, 16 USPQ2d 1934 (Fed. Cir. 1990). Accordingly, we conclude that the subject matter of the claims on appeal would have been prima facie obvious.

We have not overlooked the claimed limitation of 6-28 hours and the apparent discouragement described by German Patent 29 20 592 and Epstein of not exceeding "about 45 minutes." Epstein discourages heating for more than 45 minutes, because the viscosity of his media is said to increase. Because Epstein is collecting riboflavin from a supernatant, an increase in viscosity would decrease yield. As we pointed out in Finding 16, unlike applicant, Epstein has not provided any microscopic data to confirm the statement that "heating serves to lyse the cells." Given the time and temperature taught by Epstein, and his observation as to when the media becomes viscous, it is reasonable to conclude that

Appeal No. 93-4005
Application 07/795,158

cell lyses may not begin until after 45 minutes. Thus, although Epstein realized, and suggested, that enhanced yield would be obtained when riboflavin-producing microorganisms are lysed, there is no evidence that Epstein ever worked with cells in a lysed condition.

When we turn to the Babayan reference, which describes autolysis of various microorganisms, we do not find any discouraging statements with respect to time. In view of the different culture variables described by Babayan, those skilled in the art would have expected that gentle heating of an oxygen-deprived culture of filamentous fungi would have resulted in the complete enzymatic breakdown of the cell wall and proteins over time.

Hence, the time statements by Epstein reduce to a mere conclusion. Our appellate reviewing court recently made the observation that nothing in applicable jurisprudence requires the fact finder to credit the unsupported assertions of an expert witness. Rohm and Haas Co. v. Brotech Corp., 127 F.3d 1089, 1092, 44 USPQ2d 1459, 1462 (Fed. Cir. 1997).

Appeal No. 93-4005
Application 07/795,158

Moreover, other prior art manifestly reveals that time is a result oriented variable. See, e.g., Reyes and the discussion in our Findings 37-39.

In addition to relying on considerable prior art not of record when the appeal reached the board, we have also relied on new rationale. Accordingly, this rejection is designated as a new ground of rejection under 37 CFR § 1.196(b).

C. Additional prior art

During the course of our deliberations, we became aware of two additional publications. We call those publications to the examiner and applicant:

(1) Ozbas et al., "Comparative study of riboflavin production from two microorganisms: Eremothecium ashbyii and Ashbya gossypii," 8 Enzyme Microb. Technol. 593 (1986).

(2) Tanaka et al., "Enzymatic Hydrolysis of Yeast Cell Walls," "Susceptibilities of Isolated Cell Walls and Ascus Walls of Various Yeasts to the Actions of Bacterial Endo- β -glucanases," Symposium on Yeast Protoplasts (1967).

D. Decision

The decision of the examiner rejecting claims 3, 4 and 6 is reversed.

Appeal No. 93-4005
Application 07/795,158

A new ground of rejection under 37 CFR § 1.196(b) has been entered.

E. Time for taking action

This opinion contains a new ground of rejection pursuant to Rule 196(b) (37 CFR § 1.196(b), amended effective Dec. 1, 1997). See Notice of Final Rule, Changes to Patent Practice and Procedure, 62 Fed. Reg. 53131, 53197 (Oct. 10, 1997), reprinted in 1203 Off. Gaz. Pat. & Trademark Office 63, 122 (Oct. 21, 1997)).

Rule 196(b) provides that, "A new ground of rejection shall not be considered final for purposes of judicial review."

Rule 196(b) also provides that the applicant, **WITHIN TWO MONTHS FROM THE DATE OF ENTRY OF THIS DECISION**, must exercise one of the following two options with respect to the new ground of rejection to avoid termination of proceedings (§ 1.197(c)) as to the rejected claims:

- (1) Submit an appropriate amendment of the claims so rejected or a showing of facts relating to the claims so rejected, or both, and have the matter

Appeal No. 93-4005
Application 07/795,158

cc: KEIL & WEINKAUF
1101 Connecticut Avenue, N.W.
Washington, D.C. 20036