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D E C I S I O N
of 7 October 2003

Case Number: T 0214/00 - 3.4.2

Application Number: 87905954.1

Publication Number: 0282532

IPC: G01N 33/48

Language of the proceedings: EN

Title of invention:
Determination of biomass

Patentee:
ABERTEC LIMITED

Opponent:
-

Headword:
-

Relevant legal provisions:
EPC Art. 123(2), 56

Keyword:
"Amended independent claims admissible - Article 123(2)(3)
(yes)"
"Inventive step - yes"

Decisions cited:
-

Catchword:
-



Case Number: T 0214/00 - 3.4.2

D E C I S I O N
of the Technical Board of Appeal 3.4.2
of 7 October 2003

Appellant: ABERTEC LIMITED
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Decision under appeal: Decision of the Opposition Division of the
European Patent Office posted 23 December 1999
revoking European patent No. 0282532 pursuant
to Article 102(1) EPC.

Composition of the Board:

Chairman: A. G. Klein
Members: M. A. Rayner
V. Di Cerbo

Summary of Facts and Submissions

I. The patent proprietor has appealed against the decision of the opposition division revoking European patent 282 532 (application number 87 905 954.1, International publication number WO 88/02114). The patent relates to biomass determination.

II. In the opposition proceedings, the opposition was withdrawn and the opposition division continued to examine the case of its own motion, reference being made in the decision under appeal *inter alia* to the following documents:

E(1) "Determination of Biomass Concentration by Capacitance Measurement", Gencer et al., Biotechnology and Bioengineering, Vol. XXI, pages 1097 to 1103 (1979); and

E(3) "Biological Membranes and Tissue"; Pethig, pages 207 to 243 (1979).

The decision became focussed on an amended feature including the wording "predetermined frequency being a selected frequency at which dielectric permittivity depends substantially on the β -dispersion". Matters considered by the opposition division in its decision included the following:

Article 123(2) EPC

The opposition division was of the view that neither as a whole nor *expressis verbis* did the documents as filed provide a clear teaching and support that a frequency

is to be selected at which the dielectric permittivity depends substantially on the β -dispersion. This was the reason the division considered in the light of Article 123(2) EPC that the patent had to be revoked.

Article 84 EPC

In further comments, the division explained its view that Article 84 EPC was not satisfied because the amended feature pertained to a result to be achieved.

Article 54 EPC

If the amended feature were nonetheless considered originally disclosed and not able to be defined more precisely, then the independent claims would fulfil the requirements of Article 52(1) and 54 EPC, since document E(1) does not disclose or suggest a measurement within a frequency range wherein the dielectric permittivity depends substantially on β -dispersion.

Article 56 EPC

Starting from document E(1) and considering the other cited documents, there is no inventive step in the subject matter claimed in the independent claims. Document E(1) indicates a relationship between impedance change of a biological system and biological activity as known. Errors had been experienced in a medium containing ionisable salts. Faced with the problem of obtaining reliable results, the skilled person would consider document E(3), and thus varying frequency in the range 1 kHz to 100 MHz given in this

document for cellular materials. This corresponds to the frequency given in the patent, the subject matter claimed thus being obvious over document E(1) in view of document E(3).

III. During the appeal proceedings, a third party filed observations on the case, making reference *inter alia* to document

D1 "Dielectric Properties of Yeast Cells", Asami et al., J. Membrane Biol.28, pages 169 to 180(1976).

The third party expressed the view that one can directly and without ambiguity deduce from document D1 (figures) that suitable frequencies for measurement of biomass include frequencies of the beta dispersion

IV. The board issued a summons to oral proceedings. In a communication annexed to the summons to oral proceedings, the board *inter alia* observed that it seemed that the specific wording which the opposition division considered contrary to Article 123(2) EPC was no longer present in the claims.

V. The case of the appellant can be summarised as follows:

Requests

Maintenance of the patent on the basis of the sole request presented at the oral proceedings (amended specification).

Claims

The independent claims of the main and sole request are worded as follows:

"1. A method for the determination of biomass in a culture comprising a suspending fluid and cells, the method comprising generating a signal dependent on the dielectric permittivity of material in the bulk of the medium using electrical capacitance measurement, at a predetermined frequency, between electrodes (4) mutually spaced in the medium, said frequency being selected in the half of the $\hat{\alpha}$ -dispersion which occurs at lower frequencies but at which $\hat{\alpha}$ -dispersion is substantially insignificant such that the dielectric permittivity varies with the volume fraction of the medium enclosed by the cytoplasmic membranes of the cells, and determining said volume fraction from the permittivity dependent signal.

2. A fermentation process utilising a comprising a suspending fluid and cells, the process comprising generating a signal dependent on the dielectric permittivity of material in the bulk of the culture using electrical capacitance measurement, at a predetermined frequency, between electrodes (4) mutually spaced in the culture or a sample thereof, and providing an indication if the permittivity dependent signal differs from a predetermined value or falls outside a predetermined range, and/or altering the value of a process parameter to return the signal towards the predetermined value or the predetermined range, said predetermined frequency being selected in the half of the $\hat{\alpha}$ -dispersion which occurs at lower

frequencies but at which α -dispersion is substantially insignificant such that the dielectric permittivity varies with the volume fraction of the medium enclosed by the cytoplasmic membranes of the cells.

3. Apparatus for performing a fermentation utilising a culture comprising a suspending liquid and cells, the process comprising a fermenter (2) containing the culture, electrodes (4) mutually spaced in the fermenter (2) so as to be in contact with the culture; and means (6) for generating a signal dependent on the dielectric permittivity of material in the bulk of the culture using electrical capacitance measurement between the electrodes (4), at a predetermined frequency which is selected in the half of the α -dispersion which occurs at lower frequencies but at which α -dispersion is substantially insignificant such that the dielectric permittivity varies with the volume fraction of the medium enclosed by the cytoplasmic membranes of the cells."

Arguments

The invention solves the problem of providing a quantitative biomass determination in a culture. Although document E(1) suggests using capacitance quantitatively, it provides no clear and correct teaching on the practical validity or relationship of the measured values and bulk cell content. Document E(3) does not identify β -dispersion as the only frequency relevant for cellular material. It is important to take particular care in assessing the position of the skilled person at the priority date of the patent as there is a significant danger of using hindsight in the

present case. On the one hand there are disclosures like document E(1) relating to measurements in cultures, where many parameters could be measured, and on the other rather more academic documents like document D1 concerned with cell structure. The latter documents have a different point of view and start with a given value of cell concentration in rather sterile conditions, i.e. biomass concentration in a culture is not determined. It is not disputed that it is intuitive for a scientist that properties probably change with concentration, but this generality does not correspond to what is claimed. The patent teaches the major step involved in relation to real cultures, i.e. specific use of the lower part of the β -dispersion.

VI. At the end of the oral proceedings, the board gave its decision.

Reasons for the Decision

1. *Admissibility of the appeal*

The appeal complies with the provisions mentioned in Rule 65(1) EPC and is therefore admissible.

2. *Amendments (Article 123(2) and (3) EPC)*

2.1 The wording objected to by the opposition division is no longer present in the independent claims.

2.2 Compared to the claim as granted, the independent claims have been restricted by introduction of the wording "in the half of the $\hat{\alpha}$ -dispersion which occurs

at lower frequencies but at which α -dispersion is substantially insignificant". Support for this amendment can be found on in the documents as filed (see page 5, lines 26 et seq. of WO-88/02114). Consistent with claims 2 and 3, in claim 1 the wording "determination of biomass in a medium" is replaced by the more restricted wording "determination of biomass in a culture". Similar amendments have been effected in the introductory part of the specification.

2.3 Accordingly, the board is satisfied the amendments are in compliance with Articles 123(2) and (3) EPC.

3. *Clarity (Article 84 EPC)*

The independent claims as now amended do not define a result to be achieved but selection of a frequency in the half of the α -dispersion which occurs at lower frequencies but at which α -dispersion is substantially insignificant. The board considers dispersion clear to the person skilled in the art and observes that it is mentioned in the prior art and also explained in the patent (see the paragraph bridging columns 3 and 4). Thus in the context of the amendment made, the board is satisfied as to clarity and thus compliance with Article 84 EPC.

4. *Pertinent prior art documents*

4.1 Document E(1)

The approach employed in biomass determination is based on electrical impedance of fermentation broths and a prime objective is to develop a quantitative

relationship as means of monitoring cellular concentration in industrial fermenters. Use was made experimentally of a four prong platinum electrode, potential being measured in a frequency range of 1 to 20 kHz. The capacitance of the culture was found to increase with increasing number of yeast cells therein. In experiments where large amounts of salts are present, double layer capacitance became significant in comparison with solution capacitance. Therefore the measurement of capacitance due to microbial cells became difficult. An electrode system is being developed that can measure capacitance of microbial cultures in a medium containing ionisable salts. Document E(1) concludes by stating that a cell concentration measurement has been described that gives reliable and reproducible results when conductance of the fermentation broth is low. However when the conductivity of the suspending medium is high or when ionisable salts are present, the method fails to detect capacitance changes corresponding to changes in yeast cell concentration.

Document E(1) makes reference to document D1 as showing limiting value of dielectric constant is strongly dependent on cell volume fraction. Thus the capacitance of the medium is expected to change dependent on the number of yeast cells.

4.2 Document E(3)

Figure 7.8 shows frequency variation of relative permittivity obtained for biological tissue. A low frequency region is associated with interfacial phenomena. An intermediate frequency region is

associated with the capacitance of cell membranes, where the effect of membrane capacitance falls as the frequency increases until around 100 Mhz only the intra and extra cellular fluids dominate the dielectric properties. Relatively steady permittivity value in the range 100 to 3000 MHz is essentially governed by the water content of the tissue. Figure 7.9 shows variation of permittivity for low and high water contents.

4.3 Document D1

Figure 1 shows frequency dependency of dielectric constant of yeast cell suspensions in various volume fractions. The suspending medium is KCl, various volume fractions being obtained by dilution. Dielectric constant showed a remarkable dependence on frequency and volume fraction. The dielectric dispersions are assigned to the β -dispersion. The document goes on to explain an electrical model for yeast cells.

5. *Novelty (Article 54 EPC)*

Since the frequency given in document E(1) lies outside the β -dispersion, no disclosure of selection of the β -dispersion can be found, let alone a disclosure of the half of the $\hat{\alpha}$ -dispersion which occurs at lower frequencies but at which $\acute{\alpha}$ -dispersion is substantially insignificant. While document E(3) or D1 show for given concentrations permittivity and frequency relations including the β -dispersion, these disclosures lack a feature pertaining to "generating a signal" and subsequent features associated with biomass determination, fermentation or means therefor as recited in the independent claims. Selection of the

part of the β -dispersion claimed is not mentioned in either document. None of the other documents in the file are more pertinent to the subject matter claimed than documents E(1), E(3) or D1.

Accordingly, the board is satisfied as to the novelty of the subject matter of independent claims 1, 2 and 3.

6. *Inventive step (Article 56 EPC)*

6.1 The documents concerned with properties of biological materials do not represent the closest prior art, since in the case of document D1 concentrations are "given", and not determined, for the purpose of discussion of dielectric properties of yeast cells and in the case of document E(3) the general discussion of fluid and water content of biological tissues is further away. In agreement with the first instance, the board thus sees document E(1) as representing the closest prior art as it is concerned with biomass determination in fermenters, especially based on electrical impedance.

6.2 The problem solved by the frequency being selected in the half of the $\hat{\alpha}$ -dispersion which occurs at lower frequencies but at which $\hat{\alpha}$ -dispersion is substantially insignificant is improving quantitative biomass determination. According to document E(1) a reliable measurement of concentration is already provided by its teaching at low conductance of the fermentation broth, the skilled person is thus not motivated towards "improving" *in vacuo* but is led to start from the difficulty mentioned with capacitance determination in the presence of large amounts of salts. Contrary to opposition division, the board however considers that

just this disclosure leads away from the invention because the focus is put on the electrode structure as the way to solve the difficulty. As the skilled person expects this measure will solve the difficulty, the approach used would necessarily be tied thereto and thus there is no reason for also or alternatively considering changing frequency. Even supposing the lead to the electrode structure were to be ignored by the skilled person, why should frequency as opposed to any other process parameter or other structural feature of the cell be chosen for solving the difficulty? While the skilled person might expect some relation to exist between concentration and frequency, such a relation would also be expected between other process parameters and concentration. Nevertheless, supposing for the sake of argument that, consequent to dispersion shown for different given concentrations in document D1, the skilled person could consider an approach involving changing frequency, the view of the board is that it is not obvious that this approach would have been pursued. This is because document D1, which is taken into account in the discussion in document E(1), is, despite its showing β -dispersion, not picked up by the author of document E(1) as suggesting selecting frequency as a way towards solving the difficulty faced. This indicates to the board that the author of document E(1) did not recognise the significance of frequency in the β -dispersion as alternative to electrode construction, contrary to the suggestion of the third party that this document leads to suitable frequencies in this context. There is therefore a series of doubtful steps necessary even to reach selecting a frequency in the β -dispersion, which the board can only see being motivated by hindsight. Even then, such use of hindsight does not

- lead to a frequency in the half of the β -dispersion as specified in the independent claims.
- 6.3 The region shown in Figure 7-8 of document E(3) for cellular materials could also only be chosen as providing the solution to the difficulty identified in document E(1) using similar hindsight considerations, as in this case too there are no objective reasons for the skilled person, when faced with the difficulty defined in document E(1), to move away from the solution suggested in relation to electrode construction to frequency selection, nor any reason to expect then to choose the half of the β -dispersion specified in the independent claims.
- 6.4 The board is therefore satisfied that the subject matter of independent claims 1 to 3 is not obvious to a person skilled in the art. This conclusion is not affected by the other less relevant documents in the file. Accordingly, the subject matter of independent is considered as involving an inventive step. A similar conclusion applies to dependent claims 4 to 6 which depend from one or more of these independent claims.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent with the amended specification as filed at the oral proceedings.

The Registrar:

The Chairman:

P. Martorana

A. G. Klein