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D E C I S I O N
of 9 January 2002

Case Number: T 0795/98 - 3.3.4

Application Number: 90904261.6

Publication Number: 0461166

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Language of the proceedings: EN

Title of invention:
A method for controlling and/or monitoring biological processes

Patentee:
AKTIESELSKABET FAXE KALKBRUD

Opponent:
BioChem Technology, Inc.

Headword:
Biological process/AKTIESELSKABET FAXE KALKBRUD

Relevant legal provisions:
EPC Art. 54, 56

Keyword:
"Novelty - yes"
"Inventive step - yes"

Decisions cited:
-

Catchword:
-

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Case Number: T 0795/98 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 9 January 2002

Appellant: (Opponent)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 29 May 1998 concerning maintenance of European patent No. 0 461 166 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
S. U. Hoffmann

Summary of Facts and Submissions

I. The Appellants (Opponents) appealed the decision of the Opposition Division to maintain in amended form the European patent No. 0 461 166 filed under No. 90 904 261.6 with the title "A method for controlling and/ or monitoring biological processes".

The set of claims 1 to 20 accepted by the Opposition Division only differed from the granted set of claims in that a rearrangement of words was carried out in claim 15. The Opposition Division acknowledged novelty and inventive step of the method claims 1 to 17 and of claims 18 to 20 directed to a plant for carrying out the method of claims 1 to 17.

II. The Board summoned oral proceedings which took place on 9 January 2002. During the oral proceedings, the Board expressed doubts about the novelty of claims 18 to 20. The Respondents (Patentees) then filed granted claims 1 to 17 as sole request, claims 18 to 20 being cancelled. Claims 1 and 15 read as follows:

"1. A method for controlling and/or optimising a waste water purification process in which an aqueous system comprising biodegradable material is subjected to biodegradation by a mixed culture of microorganisms so as to obtain as a final product purified water which has a concentration of biodegradable matter which is at least 5 times smaller than in the aqueous system, which method comprises

monitoring the microbiological activity of the biological system constituted by the mixed culture of microorganisms biodegrading the biodegradable material

and/or fluctations of the activity by on-line measurement of fluorescent emission and/or variations therein for a characteristic biogenic fluorophore present in the mixed culture of microorganisms in the system upon excitation, and

controlling one or several parameters of the process by using results from the measurement as measured variable(s) in an on-line automation system."

"15. A method of quantitatively and/or qualitatively assessing the content of biodegradable material in an aqueous system comprising a mixed culture of microorganisms or fluctuations in the content, the method comprising measuring, by on-line measurement, fluorescent emission of a characteristic biogenic fluorophore present in the mixed culture of microorganisms and capable of acting as indicator(s) of the level of microbiological activity of the mixed culture of microorganisms and thereby of the amount and/or quality of biodegradable material present in the aqueous system, when irradiated with light emitted at a wavelength in the range of 250-780 nm, the fluorescence emission being detected at wavelengths in the range of 250-800 nm, and using the measured values of the fluorescence emission as basis for the assessment."

Dependent claims 2 to 14 related to further features of the method of claim 1. Dependent claims 16 and 17 related to further features of the method of claim 15.

III. The following documents are mentioned in the present decision:

(2): Zabriskie, D.W. and Humphrey, A.E., Applied and

Environmental Microbiology, Vol. 35, No. 2, pages 337 to 343, 1978,

(3): US 4 686 372,

(11): Zabriskie, D.W., Biotechnology and Bioengineering Symp., Eds John Wiley and Sons, Inc., No. 9, pages 117 to 123, 1979.

IV. The submissions in writing and during oral proceedings by the Appellants (Opponents) can be summarized as follows:

Claim 15; novelty

Document (3) disclosed a method whereby the fluorescent emission of a biogenic fluorophore (F_{420}) present in a culture comprising many kinds of microorganisms was measured as an indication of the metabolic activity of the specific kind of microorganisms in the culture producing said fluorophore (methanogens; column 2, lines 3 to 17). The range of wavelengths for emission and detection of the fluorescence overlapped that used in the method of claim 15 (column 2, line 22).

In addition, document (3) disclosed that NAD(P)H was a biological substance present in all kinds of microorganisms (column 1, lines 64 to 67), and that the optical measurement of NAD(P)H was indicative of the microorganism activity (column 1, lines 45 to 52).

Thus, the overall teachings of document (3) amounted to a teaching of a method whereby the overall metabolic activity of all kinds of microorganisms present in a mixed culture was assessed by measuring the

fluorescence of NAD(P)H. Consequently, document (3) disclosed a method which was comprised within the scope of claim 15 and destroyed the novelty of said claim.

Contrary to the Respondents' opinion, there was no convincing evidence in document (3) that adsorbance rather than fluorescence was meant to be the mean to use to measure NAD(P)H.

Claim 15; inventive step

Document (3) was the closest prior art to the subject-matter of claim 15. Starting from this document, the problem to be solved could be defined as providing a method for assessing the overall metabolic activity of a culture comprising many different microorganisms.

The solution given in claim 15 was to measure the fluorescence of a substance common to all of the microorganisms contained in said culture.
This solution was obvious since document (3) taken as a whole not only disclosed a substance common to all microorganisms (NAD(P)H) but also identified fluorescence as a suitable mean for measuring a similar kind of substance (F_{420}).

Alternatively, the solution given in claim 15 was obvious when the combined teachings of documents (3) and (2) were taken into account, as document (2) taught that in case of a monoculture used for fermentation, the fluorescence due to NAD(P)H (disclosed in document (3)) provided a sensitive index of the culture activity. As was apparent from the summary on page 337, document (2) did not discard the possibility that fluorescence could be an index of metabolic activity in

case of a mixed culture.

In document (2), (page 343, left-hand column, 2nd par.) and document (11), (page 121, discussion) which was equally concerned with the use of fluorescence (NAD(P)H) for monitoring fermentation systems, the advantage of the fluorescence method for evaluating metabolic activity was emphasized.

Claim 1; inventive step

The method of claim 1 was of a narrower scope than that of claim 15, as it amounted to applying the latter method to the control and optimisation of a waste water purification process (the range of wavelengths of fluorescence adequate to monitor the biological activity being however omitted).

The reasons given for lack of inventive step of the subject-matter of claim 15 over the teaching of document (3) equally applied to the subject-matter of claim 1, all the more so that the assessment of the metabolic activity of one kind of microorganisms in document (3) had been carried out in a methane fermentation tank for waste water treating system. It would thus readily come to the skilled person's mind to use fluorescence to assess the overall metabolic activity of all of the microorganisms involved in the waste water purification process.

V. The submissions in writing and during oral proceedings by the Respondents (Patentees) can be summarized as follows:

Claim 15; novelty

Document (3) comprised two parts: the invention per se which dealt with assessing the metabolic activity of one kind of microorganisms in a mixed culture by measuring the fluorescence of a fluorophore specific to that kind of microorganisms and a summary of background technology relative to known means of assessing metabolic activity of microorganisms. One such mean was defined in column 1, lines 45 to 52 as measuring the levels of NAD(P)H produced by the microorganism tested. Yet, adsorbance rather than fluorescence was identified as the mean to measure NAD(P)H levels, as could be inferred from column 1, lines 56 to 59 in particular wherein the necessity for the measurement to be carried out in the absence of extraneous compounds in the culture (sludge) was emphasized, which necessity only occurred if adsorbance was intended. It was also disclosed in column 1, lines 64 to 67 that NAD(P)H was present in all kinds of microorganisms.

Thus, in the first part of the document, fluorescence of NAD(P)H was not envisaged as a mean of measuring the metabolic activity of a microorganism, let alone of a mixture thereof and, in the second part of the document, the fluorescence of a fluorophore which was specific of only one kind of microorganisms was the mean of measuring the metabolic activity of said microorganism. Consequently, it was not possible to interpret the document as a whole as a disclosure of a method as in claim 15 whereby fluorescence of a compound common to all microorganisms present in a mixed culture was to be used for assessing the overall metabolic activity of said culture. The subject-matter of claim 15 was novel.

Claim 15; inventive step

Document (3) taught that the metabolic activity of one kind of microorganisms could be evaluated by measuring the fluorescence of a specific fluorophore which it produced. Yet, it did not suggest that this method could be extended to cultures comprising different kinds of microorganisms. Furthermore, although it mentioned NAD(P)H as a substance common to all types of microorganisms, it failed to mention fluorescence as a mean to measure it. It was not possible to derive the subject-matter of claim 15 in an obvious manner from such a teaching.

The argument by the Appellants that the combined teachings of document (3) and of document (2) was detrimental to inventive step was also not convincing. Indeed, although the summary of document (2) on page 337 disclosed that in a fermentation under controlled conditions, there existed a linear relationship between the log of the biomass concentration (biomass being one of the parameters determining the level of metabolic activity) and the log of fluorescence (mostly due to NAD(P)H), it was readily apparent from reading document (2) as a whole that this result had only been obtained with some pure cultures but not with all of them.

Insofar as the relationship between biomass and fluorescence varied from one organism to another, it was impossible to predict what this relationship would be in a culture comprising many different types of microorganisms and, it was even less possible to predict that there would be a relationship between the quality and/or quantity of the biodegradable material and the fluorescence of the mixed culture used to degrade it. It was the merit of the invention to have

shown against expectations that this link did exist and that, therefore, the latter (fluorescence) could be used for the evaluation of the earlier (biodegradable matter).

Document (2), page 343, left-hand column, 2nd par. expressed doubts that the relationship between biomass of pure cultures and fluorescence would be observed under conditions where the environment could not be controlled. In document (11), it was considered impossible to understand why this relationship should exist in pure cultures whereas many factors were likely to affect metabolic activity. Both these documents taught away from the subject-matter of claim 15.

Claim 1; inventive step

The same reasons which imparted inventive step to the subject-matter of claim 15 equally applied to claim 1. The fact that document (3) disclosed that the method, it described with regard to one microorganism could be carried out in the fermentation tank for waste water treatment, of course, pointed out to a waste water purification system, yet, it did not alter the fact that the possibility of controlling the waste water purification process by monitoring the overall metabolic activity of the mixed culture involved in said process via its fluorescence could not have been predicted.

VI. The Appellants requested that the decision under appeal be set aside and that the patent be revoked.

The Respondents requested that the decision under appeal be set aside and the patent be maintained on the

basis of the set of claims 1 to 17 filed at oral proceedings.

Reasons for the Decision

Claim 15; novelty

1. Document (3) discloses a method which involves measuring the fluorescence of the fluorophore F_{420} which is a substance characteristic of methanogens, as an indicator of the metabolic activity of these **specific** microorganisms (column 2, lines 3 to 17). This measurement is shown to be useful when carried out in the presence of other microorganisms (Fig.9) to identify and distinguish the methanogens from the latter. The subject-matter of claim 15 involves measuring the fluorescence of a fluorophore which is a substance characteristic of a **mixed culture** of microorganisms as an indicator of their overall metabolic activity.

2. As the invention described in document (3) is not concerned with assessing the overall metabolic activity of different kinds of microorganisms contained within the same culture, it is for this reason alone not novelty destroying to the subject-matter of claim 15.

3. However, it was argued by the Appellants that, in the light of the "Background of technology" part of document (3), the above mentioned method would be understood as a disclosure of the method of claim 15. In this part of the document, known methods of measuring metabolic activity are reviewed: mention is

made of the adsorbance method as well as of the optical measurement of NAD(P)H (column 1, lines 21 to 52). It is also disclosed that NAD(P)H is a substance existing in all kinds of microorganisms (column 1, lines 64 to 68). However, fluorescence is not mentioned as the kind of optical measurement for NAD(P)H. Neither is it contemplated to measure NAD(P)H in a mixed culture.

4. Since, firstly, it is not derivable from the first part of document (3) that fluorescence is the optical measurement to be carried out to measure metabolic activity, and, secondly, the assessment of the overall metabolic activity of a mixed culture is not disclosed in any parts of the document (although a substance common to all microorganisms is identified), the Board concludes that document (3) also does not disclose in an implicit manner the subject-matter of claim 15.

5. It was also argued that the invention according to document (3) was a specific embodiment of the method of claim 15. This, however, cannot be accepted because document (3) does not disclose that measuring the fluorescence of a substance specific for **one kind** of microorganisms in a mixed culture is an indicator of the metabolic activity of the mixed culture **as a whole**. To the contrary, the goal which is intended to be achieved is to monitor one type of microorganisms independently from the others.

6. There are no other documents on file, the teachings of which would destroy the novelty of the subject-matter of claim 15. Novelty is acknowledged.

Claim 15; inventive step

7. The closest prior art is document (3) which is concerned with the monitoring of the quantity of methane which is produced in a fermentation tank containing a mixed culture comprising many different kinds of microorganisms including methanogens. It discloses that this monitoring may be achieved through the assessment of the metabolic activity of the methanogens, which metabolic activity is shown to be directly proportional to, and, therefore, represented by, the fluorescence of a substance which **only the methanogens** produce (Figures 6 and 7, passage bridging column 6, line 65 to column 7, line 21).

8. Starting from the closest prior art, the problem to be solved may be defined as monitoring the quality and/or quantity of biodegradable material which is consumed by a mixed culture containing many different kinds of microorganisms.

9. The solution proposed in claim 15 is to achieve this monitoring through the assessment of the overall metabolic activity of a mixed culture, which assessment is done by measuring the fluorescence of a substance produced by **all microorganisms** present in said culture. The invention, thus, provides the knowledge that there exists a linear relationship between the metabolic activity of a mixed culture and the fluorescence of said culture as a whole, i.e. that for each and every kind of microorganisms present in the culture, there is a linear relationship between their metabolic activity and the fluorescence of the chosen substance providing the prerequisite for a reliable monitoring.

10. To decide whether or not this solution could have been reasonably expected by the skilled person at the

priority date, it is necessary to investigate what was the state of the art at the time regarding the relationship between the metabolic activity of microorganisms and the fluorescence of any one substance produced by them.

11. None of the documents on file describes the metabolic activity of mixed cultures. Document (2) describes a study of the above mentioned relationship in the case of three fermentations carried out separately under controlled environmental conditions with monocultures of three different microorganisms; metabolic activity being evaluated as the number of cells (biomass) present in the cultures. It is found that the linear relationship between biomass and fluorescence fails for one culture out of the three. Discussing the results obtained with the other two cultures, the authors of document (2) express doubts that the observed relationship would exist under uncontrolled environmental conditions. The same attitude is taken in document (11) (also concerned with the use of culture fluorescence for monitoring fermentation by monocultures), where it is emphasized on page 121 that: *"...culture fluorescence is a complex function of biomass concentration, cellular metabolic activity and a variety of environmental factors. Although this complexity makes a detailed understanding of the behavior of these data impossible at this time, culture fluorescence appears to provide a cumulative index of culture activity and may therefore have importance in the control of a variety of fermentation processes."*

12. Thus, in view of the prior art, it must be concluded that there is no compulsory linear relationship between the metabolic activity of a **monoculture** and the

fluorescence of a substance involved in its metabolism (document (2)), and also that such a relationship, although sometimes observed (as in document (3)), was considered surprising (documents (2) and (11)).

13. In the Board's judgment, the reliable relationship between the metabolic activity and the fluorescence showing monitoring is even more surprising in the case of a **mixed culture** where the metabolic activity of one type of microorganisms in the culture may influence that of the others present in the mixture (uncontrolled environmental conditions). It is all the more unpredictable that the fluorescence of a mixed culture reflects the quality and/or quantity of biodegradable material consumed.

14. The Appellants pointed out that on the basis of the summary of document (2) where the correlation between culture fluorescence and biomass concentration is disclosed without mentioning for which kind of cultures it was observed (mono or mixed), the person skilled in the art would not have discarded the possibility that it did exist in case of mixed cultures. This argument, however, is not convincing because it is expected from a person skilled in the art that he/she will read an apparently relevant document, as a whole and will interpret any of its parts taken in isolation in the light of the overall teaching provided. In the present case, the summary would not suggest to the skilled person the above mentioned correlation in case of mixed cultures for the reasons given in point 11 above.

15. It was also pointed out that on the basis of document (2), page 343: *"Evidence suggests that culture fluorescence is a measure of culture metabolic activity*

i.e. the product of biomass concentration and the relative rate of metabolic activity of each cell. It is therefore reasonable to speculate that culture activity may prove to be a more important parameter to monitor and regulate than biomass concentration when optimizing and controlling fermentations..."
and on the basis of document (11), page 121: *culture fluorescence appears to provide a cumulative index of culture activity and may therefore have importance in the control of a variety of fermentation processes."* , it would have been obvious for the skilled person to consider fluorescence as an important parameter to measure in the case of mixed cultures as well.

16. These statements concern fermentations i.e. processes carried out with monocultures and, besides, they are of a quite speculative nature, such terms as "suggests" "speculate" "may prove" "appears" "may have" being used. Furthermore, they cannot be read out of the context in which they are written. And this context, as already mentioned above, leads to the conclusion that the results obtained with some microorganisms are not extendable to all kinds of microorganisms in any environmental conditions. In the Board's judgment, they are not sufficient to render the subject-matter of claim 15 obvious.

17. The subject-matter of claim 15 and dependent claims thereof is inventive.

Claim 1; inventive step

18. The method of claim 1 involves measuring the fluorescence of a characteristic fluorophore present in a mixed culture of microorganisms used to degrade the

biodegradable material present in waste water in order to monitor the microbiological activity of the biological system constituted by said mixed culture ie. the metabolic activity of **all** of the microorganisms present in the culture. This method like that of claim 15 implies that there exists a linear relationship between metabolic activity and fluorescence. It was found in points 10 to 13 above that such a relationship could not reasonably be expected in light of the state of the art: documents (3), (2) and (11) and, thus, imparted inventive step to the subject-matter of claim 15. The same must be true of claim 1.

19. The Appellants pointed out that in contrast to claim 15, claim 1 does not mention ranges of wavelengths for emission and detection of fluorescence. This is, indeed, the case. Yet, as the very wide range of wavelengths mentioned in claim 15 is not the reason why the subject-matter of said claim was found to be inventive, failing to mention it in claim 1 cannot take away inventive step.

20. It was also argued that the process disclosed in document (3) of measuring methane production by methanogens had been defined as possibly taking place in a methane fermentation tank for the **waste water treatment**, making obvious the monitoring of the metabolic activity of a mixed culture in a waste water tank. Thereagain, the Board must disagree for the same kind of reasons as in point 19, above: that inventive step is not due to the circumstances in which the claimed process is taking place and, therefore, whether or not these circumstances are mentioned in the art is not likely to affect it.

21. The requirements of Article 56 EPC are fulfilled.

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 with the order to maintain the patent on the basis of claims 1 to 17 filed during the oral proceedings and an amended description page 3 to 23 filed during the oral proceedings and Figures 1 to 12 as granted.

The Registrar: The Chairwoman:

P. Cremona U. Kinkeldey