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(71)(72) Applicants and Inventors: MATTIASSON, Bo [SE/SE]; Trädgårdsmästaren 26, S-222 48 Lund (SE). HÄGERDAL, Bärbel [SE/SE]; Mätaregränden 10, S-222 47 Lund (SE). ALBERTSSON, Per-Åke [SE/SE]; Warholms väg 6 c, S-222 65 Lund (SE).

(74) Agent: CARMINGER, Lars; Carminger & Uusitalo Patentbyrå AB, Kungsgatan 38, S-111 35 Stockholm (SE).

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(54) Title: BIOLOGICAL AND CHEMICAL CONVERSION PROCESSES IN LIQUID PHASE-SYSTEM

(57) Abstract

Method for carrying out biological and chemical conversion processes which require the presence of one or more catalytically active substances, such as enzymes, microorganisms, cells, organelles, cell homogenates, and organic or inorganic catalysts. The method is carried out in a liquid system consisting of at least two aqueous phases (F1, F2) so selected that the catalytic substance (K) is enriched in one (F1) of the phases, while the starting substrate (S) of the process is distributed in both phases or, advantageouly, is enriched in the same phases as the catalytic substance, and the process product (P) is distributed in both phases or, advantageously, is enriched in the other phase (F2). During the process, the system is agitated to finely disperse one phase in the other. The process product can be recovered from the other phase, either by ceasing to agitate the system and allowing the phases to separate, or by maintaining dispersement of one phase in only a part of the other phase and continuously tapping off the upper part of the other phase for removal of the process product therefrom and then returning the removed phase part to the agitated part of the liquid system. Advantageously, the other phase (F2) of the liquid system has a substantially larger volume than the mentioned one phase (F1). The method can be applied with gaseous, low-molecular, macromolecular and particulate starting substrates, which may be soluble or insoluble in the system. The method according to the invention affords particular advantages when carrying out enzymatic conversion reactions requiring the presence of a coenzyme. In this case, the coenzyme and an enzyme operable to regenerate the coenzyme are also introduced to the system, the two phases of which are selected so that the coenzyme and the further enyzme are also enriched in the same phase as the enzyme requisite to the conversion reaction.

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BIOLOGICAL AND CHEMICAL CONVERSION PROCESSES IN LIQUID PHASE – SYSTEM

The present invention relates to a method of carrying out biological and chemical conversion processes which require the presence of at least one catalytically active substance, which may be, for example, enzymes, micro-
5 organisms, whole cells, cell homogenates, organelles, or organic or inorganic catalysts.

The realization of such conversion processes on a large scale is encumbered with several problems which have hitherto been found difficult to resolve in a satisfactory
10 manner. One such problem encountered is that of retaining the catalytic substance in the process chamber, so as to avoid losing said substance, which in many cases is expensive, or to obviate the need of providing complicated and expensive additional process steps for separating the catalytic substance
15 from the process products removed from said process chamber. In certain cases it is also desired to prevent the catalytic substance from being included in the desired final product of said process as an undesirable impurity. Attempts have been made to solve this problem by immobilizing the catalytic sub-
20 stance, by binding said substance to a carrier in some suitable manner so that said substance can be held in the process chamber and the aforementioned disadvantages eliminated, thereby enabling the process to be effected as a continuous process. This immobilisation of the catalytic substance, however, gives
25 rise to other problems. It has been found, for instance, that the activity of the immobilized catalytic substance is impaired with respect to the starting substrate of the process, due to diffusion restrictions and steric hindrance, which becomes particularly noticeable with macromolecular or particulate
30 starting substrates. Thus, hitherto it has not been found possible to carry out biochemical conversion processes with macromolecular or particulate starting substrates and while

using immobilized catalytic substances, such as enzymes and microorganisms for example.

One problem which is particularly difficult to resolve exists when carrying out biochemical conversion processes with the use of a catalytically active enzym which is not able to function unless in the presence of a coenzyme. In this case, it is not only necessary for the enzyme and the coenzyme to be retained in the process chamber, but also for the coenzym to be continuously regenerated over the duration of the process, so that it can be returned to its originally active configuration with respect to the enzyme. Hitherto, it has not been possible to solve this problem in any way other than to use whole immobilized cells which contain both the enzyme required for the conversion process and the coenzyme, and in which regeneration of the coenzyme can also take place. Because of the aforementioned reasons, however, such immobilized cells cannot be used in conjunction with macromolecular or particulate starting substrates; and such cells also have the serious disadvantage whereby they result in a plurality of mutually different enzyme-activities in addition to the activity specifically desired for the conversion process, meaning that a large number of undesirable secondary reactions can take place.

A further serious problem often occurring in biochemical conversion processes of the aforementioned kind, is that the end product of the conversion process, or intermediate products formed in different stages in a conversion process comprising a number of stages, often has a strong inhibiting effect on the process or the various process stages. In consequence hereof it is necessary to hold the concentration of the end product and such intermediate products as may be formed at a low level during the process, in order to obtain a high process yield and to enable high concentrations of starting substrate and catalytic substance to be used.

The object of the present invention is to provide a novel and useful method of carrying out biochemical conversion processes of the aforedescribed kind in which the aforementioned problems can be resolved in a satisfactory manner.

The characterizing features of the method according to the invention are set forth in the accompanying claims.

The invention will now be described in more detail with reference to the accompanying drawing, in which

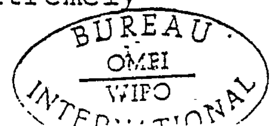
5 Figures 1A, 1B och 1C illustrate schematically a non-continuous conversion process carried out in accordance with the method of the invention;

Fig. 2 illustrates schematically a conversion process carried out continuously in accordance with the method of the
10 invention;

Figures 3A and 3B illustrate schematically a conversion process using a coenzyme-dependent enzyme when applying the method according to the invention; and

Figures 4, 5 and 6 are diagrams illustrating the results
15 obtained in tests carried out when applying the method according to the invention for converting cellulose to ethanol.

In accordance with the invention, the conversion process is carried out in a liquid system including at least two liquid phases which are so selected that the catalytic substance used
20 is enriched in one of said phases. Figure 1A illustrates schematically one such biphase liquid system, including a bottom phase F1 and a top phase F2. The biphase system is so selected that the catalytic substance K used, for example an enzyme present in solution, is enriched in the bottom phase
25 F1. The starting substrate S for the conversion process may be uniformly distributed in the biphases F1 and F2, although there may be selected to special advantage a system in which also the substrate S is enriched in the same phase F1 as the catalytic substance K, as illustrated in Figure 1A. In this case, the top
30 phase F2 contains neither the catalytic substance K nor the starting substrate S. When carrying out the conversion process according to the invention, the liquid system is agitated so that the top phase F2 becomes finely dispersed in the bottom phase F1, as illustrated schematically in Figure 1B. The con-
35 version process continues in the resultant dispersion or emulsion, within the fine droplets of the bottom phase F1, as illustrated schematically in Figure 1C which shows one such droplet. The starting substrate S and the catalytic substance K are both isolated in said droplets and are in extremely



intimate contact with each other, thereby enabling the process to continue without diffusion limitations or steric hindrance, even though the starting substrate should be macromolecular or particulate. In accordance with the invention, the two phases F1 and F2 in the liquid system are so selected that the product P formed in the conversion process is also distributed to the top phase F2, as illustrated schematically in Figures 1B and 1C. Thus, the product P passes to the top phase F2 as said product is formed in the conversion process taking place in the finely divided droplets of the bottom phase F1. In this way, it is pssible to maintain a low concentration of the product P in the bottom phase F1, so as to enable any inhibiting effect of the product on the conversion process to be eliminated or at least greatly restricted. As illustrated schematically in Figure 1, this is particularly the case when the biphase liquid system is such that the top phase F2 has a substantially much greater volume than the bottom phase F1, for example a volume which is ten times greater than the volume of said bottom phase. The distribution of the product P in the two phases F1 and F2, i.e. the concentration of the products P in the two phases, is not, in the majority of cases, appreciably affected by the ratio between the respective volumes of the two phases. Thus, it is possible in this way for a much larger amount of product P to be formed without the concentration of said product in phase F1 increasing to a value which would cause inhibition of the process. Consequently, it is possible to use the starting substrate and the catalytic substance in high concentrations and to achieve a high process yield at the same time. This favourable and sought after result can be still further improved if, in accordance with a particularly advantageous embodiment of the invention, the phases of the liquid system are selected so that the product P formed is enriched in the top phase F2, so that the concentration of product P is much greater in the top phase F2 than in the bottom phase F1, in which the conversion process continues. In this way, the concentration of product P in the bottom phase F1 can be kept at such a low level as to have substantially no inhibiting effect on the process. The transition of product P from the bottom phase F1 to the top phase F2 becomes very effective when maintaining the bottom phase F1 in a finely dispersed

state in the top phase F2, such as to provide a very large transition interface between the two phases.

When the conversion process has terminated, agitation of the system is stopped, so as to enable the two phases F1
5 and F2 to separate. In this liquid system, the catalytic substance K still remains concentrated in the bottom phase F1 together with any remaining, non-converted residues of the starting substrate S, while, if the biphase system is such that the product is partitioned to the top phase F2, a large
10 part of or substantially all the formed product P is present in the top phase F2. The top phase F2 can now be removed from the process chamber and treated in a suitable, conventional manner for recovering the product P from said phase.

Liquid systems which comprise two or more phases and
15 which can be used when carrying out the method according to the invention are, in themselves, well known and exhaustively described in the Literature. Thus, a biphase system which can be used when practicing the invention comprises mixtures of water and two mutually different polymers, which in this
20 respect may both be non-ionic polymers or polyelectrolytes or a non-ionic polymer and a polyelectrolyte. Examples of such biphase systems are dextran-polyethylene glycol-water, dextran-Ficoll-water, dextran-methylcellulose-water, dextran-polyvinylalcohol-water, Na dextran sulfate-polypropylene
25 glycol-water, Na carboxymethyldextran-polyethylene glycol-water with an addition of NaCl, Na-dextran sulfate-Na carboxymethyldextran-water. A useable biphase system may also comprise a mixture of water, a polymer and a component of low molecular weight, for example a salt. Examples of such
30 systems are polypropylene glycol-potassium phosphate-water, polyethylene glycol-potassium phosphate-water, polyethylene glycol-ammonium sulfate-water. A further account and comprehensive exemplification of aqueous biphase systems and polyphase systems which can be used in accordance with the
35 invention is given in "Partition of Cell Particles and Macromolecules", Per-Åke Albertsson, Wiley-Interscience, for example Tables 2.1 and 2.2 on pages 22, 23 and 25, and table 10.3 on page 261. Thus, there is a very large number of systems to chose from when selcting a liquid system which is suitable

both with respect to the availability and price of the phase components and with respect to the requirements placed on the conversion process in question. Because of the high water content of these aqueous biphase or polyphase systems, the
5 systems may dissolve proteins and are in general more ameniable to biological materials, such as enzymes, microorganisms and cells used as catalytic substances. The surface tension of the interface between the different phases is also very small, which means that the phases can be maintained finely mixed
10 with each other by agitation, without the energy consumed by said agitation becoming too significant.

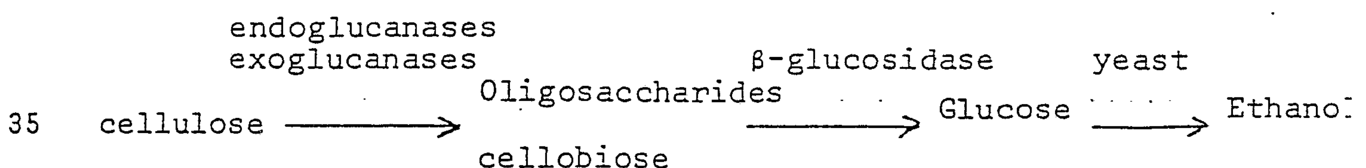
As mentioned in the aforegoing, the method according to the invention affords particularly important advantages over previously known methods when using macromolecular or parti-
15 culate starting substrates. It will be understood, however, that the method according to the invention can also be applied in conjunction with the use of different starting substrates, for example gases or low molecular, soluble or insoluble substances. The product formed in the process may also be of a
20 different kind, such as a gas, a low-molecular, macromolecular or particulate substance, and may be soluble or insoluble. Naturally, mixtures of different starting substrates may also be used, and mixtures of different process products may be produced.

25 The method according to the invention may be applied to particular advantage for carrying out continuous conversion processes. In this respect, as schematically illustrated in Figure 2, there can be used a reactor vessel 1 having a lower part 1a, which serves as a mixing chamber and in which the two
30 phases of the liquid system are maintained intimately mixed by agitation, and an upper part 1b, in which no stirring or agitation takes place and in which the two phases thus separate from each other so that only the top phase F2 is present in at least the uppermost region of the part 1b. This top phase F2
35 is removed, either intermittently or continuously, from the upper part of the reactor vessel through an outlet 2, and is passed to a treatment unit 3, in which the formed process product can be removed from the top phase F2 in a suitable

manner, said top phase then being returned to the lower part 1a of the reactor vessel through an inlet 4. The process starting substrate may be supplied to the lower part 1a of the reactor vessel in a suitable manner, not shown in detail.

5 As before mentioned, the method according to the invention can afford particularly important advantages over previously known methods when carrying out biochemical conversion processes with the use of an enzyme whose activity is dependent upon the presence of a coenzyme. As illustrated in 10 Figures 3A and 3B, in such a process, which may be carried out intermittently or, to advantage, continuously, there is added to the biphase liquid system, in accordance with the invention, not only the starting substrate S_1 for the conversion process and the enzyme E_1 requisite therefor, but also the requisite 15 coenzyme CE and a further enzyme E_2, able to co-act to produce the necessary regeneration of the modified coenzyme CE^x, so that said enzyme returns to its original configuration CE. In this case the phase system is selected so that both the enzyme E_1 and the coenzyme CE and the further enzyme E_2 are partitioned 20 to the bottom phase F1. The product formed in the desired conversion process is designated P_1 in Figure 3B, while the reference S_2 identifies a substrate requisite for regenerating the coenzyme, and the reference P_2 identifies a product formed when regenerating the coenzyme under the influence of the 25 enzyme E_2. It may be necessary also to add the substrate S_2 to the liquid system, and the phases of the liquid system may, if so is suitable, be selected so that the substrate S_2 is partitioned to the bottom phase F_1 and that both products P_1 and P_2 are partitioned to the top phase F_2.

30 The invention will hereinafter be further illustrated as applied to a process for the enzymatic degradation and conversion of particulate cellulose to ethanol. This process proceeds in accordance with the following reaction chain:



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In this chaih of reactions, the end product, ethanol, has a highly inhibiting effect on the preceding reaction stages, while the intermediate product glucose, and still more the intermediate product cellobiose, have an inhibiting effect on respective preceding reaction stages. Tests have been carried out in accordance with the invention both on the first part of the reaction chain, namely the enzymatic degradation of cellulose to glucose, and on the latter part of the reaction chain, namely the fermentation of glucose to ethanol, and also on the complete reaction chain from cellulose to ethanol.

Preparation of a biphasic system

The tests were carried out in an aqueous biphase system prepared by mixing a volume of Dextran T-40 (16 % weight/weight) (from Pharmacia Fine Chemicals, Uppsala) dissolved in a 0.1 M sodium acetate buffer, pH 4.5, with 5 volumes of PEG-6000 (12 % weight/weight) (polyethylene glycol having a molecular weight of 6000 from Union Carbide, N.Y., USA) dissolved in the same buffer.

Test 1: Fermentation of glucose to ethanol

The fermentation of glucose to ethanol when using baker's yeas was investigated, by adding 100 mg of yeast to 60 ml of the biphase system prepared in accordance with the above, and suspending said yeast in the system, after which 0.5 grams of glucose was added. The system was agitated by means of a magnetic agitator at a temperature of 30°C and samples (0.5 ml) were taken from the mixed phase system at regular intervals. These samples were centrifuged and the top phase analysed. The result can be seen from the diagram in Figure 4, where curve A shows the concentration of ethanol (mg/ml) in the top phase as a function of the reaction time (hours).

Test 2: Enzymatic degradation of cellulose to glucose

The enzymatic degradation of cellulose to glucose was investigated by adding to 60 ml of the biphase system prepared in accordance with the above 0.5 gram of cellulose (Solka Floc 200 from Brown Company, N.H. USA), 100 mg of cellulase (SP 122) (endoglucanases and exoglucanases from Tricoderma Reesei; Nova AS, Copenhagen, Denmark) and 10 mg of β-glucosidase

(E.C.3.2.1.21) (prepared from sweet almonds by P-L Biochemicals Wis., USA). The system was treated in the same manner as that recited in test 1. The result can be seen from the diagram in Figure 5, where the curve B shows reducing sugars (mg/ml) and
5 the curve C glucose (mg/ml) in the top phase as a function of time (days).

Test 3: Bioconversion of cellulose to ethanol

The whole reaction sequence from cellulose to ethanol was investigated by adding 100 mg yeast, 100 mg cellulase,
10 10 mg β-glucosidase and 0,5 g cellulose to 60 ml of the biphase system prepared in accordance with the above. Same conditions were used as in tests 1 and 2. The result can be seen from the diagram in Fig. 6, where curve D shows ethanol (mg/ml), curve E shows reducing sugars (mg/ml) and curve F
15 shows glucose (mg/ml) in the top phase as a function of time (days).

Discussion of the test results

As mentioned in the foregoing, it is important when carrying out the method according to the invention that the
20 catalytically active substances are enriched in one of the phases. Consequently, there was first investigated the partition coefficients of the enzymes and the yeast cells, i.e. the concentration in the top phase relative to the concentration in the bottom phase. It was found that the
25 yeast cells were strongly partitioned to the bottom phase (partition coefficient $K < 0.5$) and that the β-glucosidase was also partitioned to the bottom phase (partition coefficient $K = 0.25$). On the other hand, the cellulases were more uniformly distributed between the two phases when they were
30 present in a pure system, i.e. in the absence of cellulose in the system. When cellulose is added, however, the cellulases bind to the cellulose fibres and the agregate is found in the bottom phase. Thus, when cellulose is present in the system both the enzymes and the yeast cells are con-
35 centrated in the same phase as the cellulose substrate, i.e. in the bottom phase.

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The ability of the yeast cells to convert glucose to ethanol in the biphase system used in accordance with the invention was investigated in Test 1. As will be seen from curve A in Figure 4, there was obtained in the top phase after 24 hours approximately 2.5 mg/ml of ethanol. If a uniform distribution of glucose between the two phases is assumed, the initial concentration of glucose in the system was 8.3 mg/ml. If the ethanol is also uniformly distributed between the two phases, a complete conversion of glucose to ethanol would thus result in 4.25 mg/ml ethanol. Thus, after 24 hours the amount of ethanol obtained in the test was about 57 % of the theoretical yield.

The enzymatic saccharification of cellulose to glucose in the biphase system used in accordance with the invention was investigated in Test 2. Because of the high inhibiting effect of primarily cellobiose, the enzymatic saccharification of cellulose is generally characterized by a high initial rate, whereafter the process of saccharification continues very slowly. The curve C in Figure 5 shows, however, that in the test carried out according to the invention the glucose content increased constantly during the four days over which the experiment was continued. At the same time the amount of reducing sugars decreased still more strongly, which shows that substantially equal quantities of glucose and cellubiose were produced in the system. Both the amount of glucose and the amount of reducing sugars produced per unit of time decreased slowly, which indicates that inhibiting concentrations of both glucose and reducing sugars had accumulated during the latter part of the test. As a result of the favourable distribution in the biphase system used, i.e. catalysts and substrates concentrated in one phase while the products are more uniformly distributed, and the ratio between the volumes of the two phases, i.e. the top phase has a volume which is five times greater than the bottom phase, large quantities of the products, however, passed to the top phase. Continuous removal of the products from the top phase would apparently provide a still more favourable system. The high concentrations of reducing sugars compared with the glucose

concentration confirms that the β-glucosidase activity was also subjected to product inhibition.

The complete conversion process from cellulose to ethanol using the biphase system according to the invention was investigated in Test 3. The result of this test, illustrated in the diagram in Figure 6, clearly shows that the amount of yeast added was excessive compared with the amount of glucose present, i.e. the enzymatic saccharification of cellulose to cellubiose and glucose constituted the rate-limiting reaction stage. Thus, less than 0.1 mg/ml glucose was present in the system during the whole of the test. This fact is also apparent when making a comparison between the curve D in Figure 6 and the curve A in Figure 4, which shows that when converting pure glucose to ethanol there is obtained approximately twice as much ethanol as when converting glucose simultaneously produced from cellulose. A comparison between curve C in Figure 5 and curve E in Figure 6 shows that the presence of yeast effectively reduces the glucose concentration of the system, thereby eliminating the product inhibiting effect of the β-glucosidase activity. This is also clear from the fact that the cellubiose concentration (estimated as reducing sugars, curve E in Figure 6) passes a maximum and then decreases to a constant level of about 0.5 mg/ml. The fact that at this maximum level of concentration the reducing sugars are approximately ten times greater than the glucose concentration indicates a poor β-glucosidase activity.

The test was carried out over five days, during which no inhibiting effect of the ethanol could be observed. The ethanol production was substantially linear during the whole of the test period. The final value of about 5 mg/ml indicates that a practically complete conversion of cellulose to ethanol had been reached at the end of the test, assuming an equal distribution of ethanol in the system.

Thus, the tests carried out show that a bioconversion of cellulose to ethanol in a biphase system in accordance with the present invention is very effective. Such a biphase system also enables the ethanol formed to be removed continuously from the top phase of the system, whereby a continuous transfer

of ethanol from the bottom phase to the top phase is obtained, so that inhibiting concentrations of ethanol are prevented from occurring in the bottom phase, where the bioconversion process takes place. Conventional batchwise processes for manufacturing ethanol from cellulose by simultaneous saccharification and fermentation of the resultant glucose to ethanol have shown that the enzyme activity for cellulose degradation is inhibited to 50 % already at an ethanol concentration of 3 %.

In the following the method according to the invention will be exemplified with a number of additional examples.

Example 1. Production of acetone and butanol with the use of bacteria Clostridium acetobutylicum

A biphase system was used consisting of 6 % (w/w) Dextran T-40 as bottom phase and 25 % (w/w) PEG 8000 (polyethylene glycol with molecular weight 8000) as top phase. The volume ratio of the top phase to the bottom phase was 6:1. To this phase system were added 40 g/l glucose as substrate and 10 g/l peptone together with 10 g/l yeast extract as nutrition medium for the bacteria Clostridium acetobutylicum, ATCC 824. The temperature was 35°C.

The bacteria were enriched in the bottom phase, whereas the substrate and the nutrition medium were distributed in both phases.

After about 30 hours the bacteria had produced 8 g/l butanol and 3 g/l acetone to be compared with 6 g/l and 2 g/l, respectively, if no biphase system is used.

Butanol and acetone can be distilled from the top phase and if more sugar is added to the system the production will start again. This will happen also if the top phase is replaced with a new top phase without any additional nutritive medium and more sugar is added to the system.

Example 2. Production of polymer with the use of bacteria

If the fermentation in Example 1 is left to continue on its own, the butanol is after 4 days converted to a polymer,

which is not yet fully defined but which seems to be poly-hydroxy butyric acid. The reason for this conversion is believed to be that the bacteria are present in an environ-ment having a reduced water activity whereby their meta-
5 bolism is changed.

Example 3. Production of penicillin

A biphase system was used consisting of 6 % (w/w) Dextran T-40, 7.5 % (w/w) PEG 8000 and 0,3 M TRIS buffer up to 100 % (w/w), pH 7.8.

10 A continuously operating reactor of the general type illustrated in Fig. 2 was used. The reactor volume was 6 ml, of which volume 3 ml was agitated whereas the remaining 3 ml was maintained unagitated. The volume ratio of the top phase to the bottom phase was 3:1. The bottom phase included 0,5 ml
15 of a solution of penicillin acylase which was partitioned to the bottom phase. The top phase was pumped into the reactor at a rate of 1,8 ml/h.and included 70 g/l benzyl penicillin, which in the reactor was distributed in both phases. The reactor temperature was 37°C. The output flow from the
20 reactor contained 14 g/l of 6-APA.

Example 4. Hydrolysis with the use of inorganic catalyst

A biphase system was used consisting of 6 % (w/w) Dextran T-40 and 7.5 % (w/w) PEG 8000. The volume ratio top
25 phase to bottom phase was 3:1.

To this system were added 0,1 g/ml of the superacid NAFION® (Dupont, USA) as catalyst, which was partitioned to the bottom phase, 5 % (w/w) cellobiose as substrate and 0,1 % (w/w) NaCl. Within 4 days the cellobiose was converted
30 to 1,2 % (w/w) glucose.

Example 5. Biphase system in which one phase is also substrate

A phase system was used consisting of 15 % (w/w) PEG 8000 and 15 % (w/w) starch, which had been geled at 73°C in the presence of 0,6 % (v/w) of the amylase enzyme Termamyl®

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(NOVO A/S, Copenhagen). The bottom phase contained also 10% (w/w) of yeast. The initial volume ratio top phase to bottom phase was 3:1. The starch was converted to 20 g/l ethanol.

5 Example 6. Use of enzyme and coenzyme

A biphase system was used consisting of 6 % (w/w) PEG 20000 (molecular weight 20000), 10 % (w/w) Dextran T-10 (molecular weight 10000) and 0,03 M, pH 8,8, pyrophosphate buffer. The volume ratio top phase to bottom phase was 3:1.

10 The enzyme was alcohol dehydrogenase, 50 units/ml in the bottom phase, and the coenzyme was NAD^+, 8 mM. As substrate was used 200 mM ethanol, which was converted to 100 mM acetaldehyde. For the regeneration of the coenzyme one could use either a second substrate in the form of 15 glutaraldehyde, at least 200 mM, or a second enzyme in the form of alamine dehydrogenase, 50 units/ml, or a flavine in the form of flavinemononucleotide, 12 mM.

Example 7. Production of the enzyme amylase with the use of *Bacillus subtilis*

20 The same phase system as in Example 4 was used. As substrate one used 20 g/l lactose and as nutritive medium 10 g/l peptone and 10 g/l yeast extract together with mineral salts.

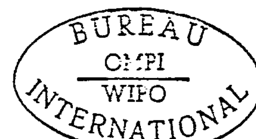
In this system *Bacillus subtilis* produced the enzyme 25 amylase after about 48 hours. The enzym was partitioned (enriched) to the top phase.

Example 8. Production of acetic acid with the use of *E. coli*

The same phase system as in Example 4 was used.

30 As substrate one used 30 g/l glucose. The nutritive medium was chosen according to Jano et al, J. Ferment. Technol., Vol. 58, No. 3 (1980), p. 259.

In this system *E. coli* produced about 5 g/l acetic acid from 30 g/l glucose. The acetic acid was partitioned 35 (enriched) to the top phase.



Example 9. Production of ethanol with the use of *Thermoanaerobacter ethanolicus*

The same phase system as in Example 4 was used.

As substrate one used not more than 10 g/l of a
5 sugar chosen according to Wiegel J., Ljungdahl L.G., Arch. Microbiol., 128 (1981), p. 343. The nutritive medium was selected according to Wiegel J., Ljungdahl L.G., Rawson J.R., (1979) J. Bacteriol. 139:800-810.

As in this system the bacteria are partitioned (enriched)
10 to the bottom phase, whereas the sugar is distributed in both phases, a larger total amount of sugar can be used in the system and converted to ethanol, without any inhibiting effect on the activity of *Th. ethanolicus*, due to a high sugar concentration.

15 Example 10. Biological conversion in a polymer-salt system

A biphase system was used consisting of 13,5 % (w/w) PEG 4000, 13,5 % (w/w) $MgSO_4 7H_2O$ and 100 mM TRIS, HCl buffer, pH 7,0. The volume ratio top phase to bottom phase was 3:1.

20 As substrate one used 14 mM phenol, 0,8 mM amino antipyrine and 1 mM H_2O_2. As enzyme one used peroxidase, which was partitioned (enriched) to the bottom phase.

In this system phenol and amino antipyrine are converted to a conjugated aromatic called quinemine, which is par-
25 titioned to the top phase. By means of this process it is possible to make phenol in e.g. waste water harmless.

Example 11. Production of ethanol with the use of *Zymomonas sp.*

The same phase system as in Example 4 was used.

30 As substrate one used 10-25 % (w/w) glucose and the nutritive medium was selected according to Rogers P.L., Lee K.J., Tribe D.E. (1979) Biotechnol. Letters 4, p. 165-170 and p. 421-426.

The use of the biphase system prevents the inhibiting effects of the substrate glucose as well as the product

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ethanol.

It will be seen from the aforegoing that the method according to the present invention can be applied to great advantage when carrying out a number of different biological
5 and chemical conversion processes. Although one of the advantages with the method according to the invention is that it is possible to use non-immobilised catalytic substances, such as enzymes and microorganisms for example, and despite this retain these catalytic substances in the process
10 chamber, it will be seen that there is nothing to prevent the invention from being applied while using immobilised catalytic substances, if so desired. The biphase or polyphase liquid system used has been exemplified in the aforegoing with reference to a system consisting of aqueous phases,
15 although it will be understood that systems based on liquids other than water can also be used if said liquids are compatible with the starting substrate, catalytic substance and products present in the process in question.

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Claims

1. A method of carrying out a biological or chemical conversion process which requires the presence of at least one catalytically active substance, characterized in that the process is effected in a liquid system in which a starting substrate (S) for the process and the catalytic substance (K) are introduced and which comprises at least two liquid phases (F1, F2) so selected that the catalytic substance (K) is enriched in one of said phases (F1), at least part of the starting substrate (S) is present in said one phase (F1), and the process product (P) is partitioned also to the other of said phases (F2); and in that said liquid system is agitated so that said one phase (F1) is held finely dispersed in said other phase (F2).

2. A method according to claim 1, characterized in that the two phases are selected so that the process product (P) is enriched in said other phase (F2).

3. A method according to claim 1 or claim 2, characterized in that the two phases are selected so that the starting substrate (S) is enriched in said one phase (F1).

4. A method according to any one of claims 1 - 3, characterizedin that said other phase (F2) has a substantially greater volume than said one phase (F1).

5. A method according to any one of claims 1 - 4, characterized in that after the process is completed, agitation is discontinued and the two phases allowed to separate, whereafter said other phase (F2) is treated for the recovery of the process product (P) therefrom.

6. A method according to claim 4, characterized in that agitation of the liquid system is effected in a manner such that said one phase (F1) is held dispersed in only a portion (1a) of said other phase (F2), and in that part of the

remaining portion (1b) of said other phase (F2), is removed from the process chamber, either intermittently or continuously, and treated for the removal of process product from said part, and then returned to the process chamber.

7. A method according to any one of claims 1 - 6, for carrying out a conversion process in which the catalytic substance is an enzyme (E_1) which requires the presence of a coenzyme (CE), characterized in that the coenzyme (CE) and a further enzyme (E_2) capable of regenerating said coenzyme are also added to the liquid system, the two phases of which are selected so that the coenzyme (CE) and said further enzyme (E2) are also enriched in said one phase (F1).

8. A method according to any one of claims 1 - 7, characterized in that the two phases of said liquid system comprise two mutually different aqueous polymer solutions.

9. A method according to any one of claims 1 - 7, characterized in that the two phases of said liquid system comprise an aqueous polymer solution and an aqueous salt solution.

Fig.1A

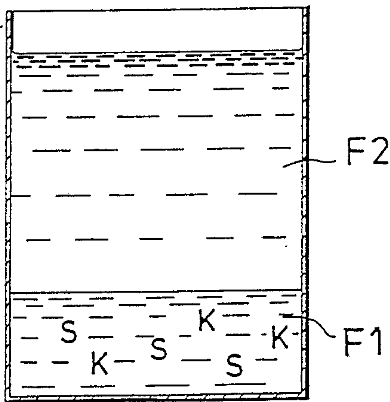


Fig.1B

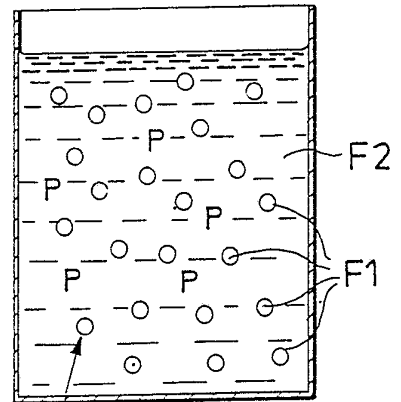


Fig.1C

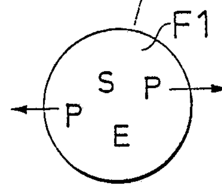


Fig.2

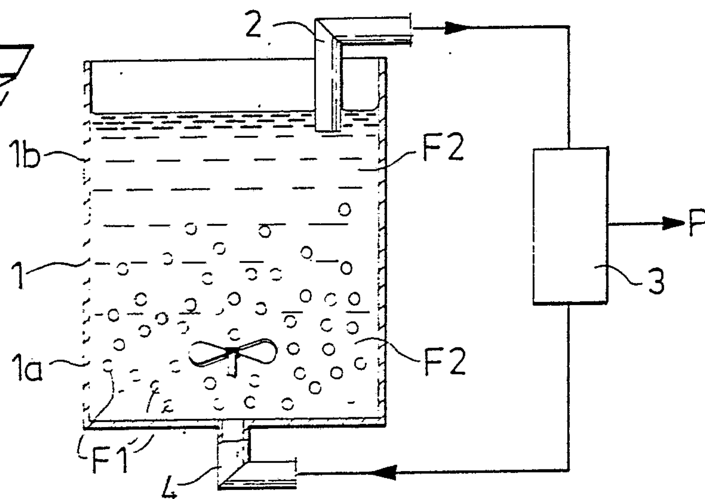


Fig.3A

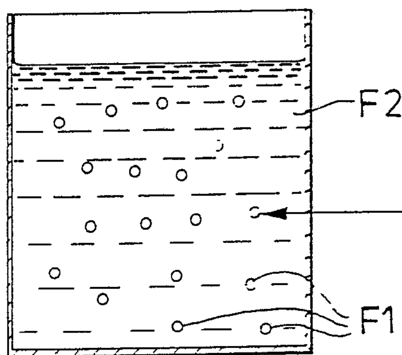
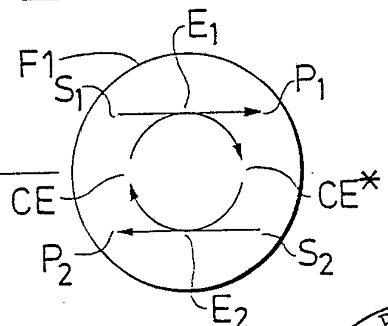


Fig.3B



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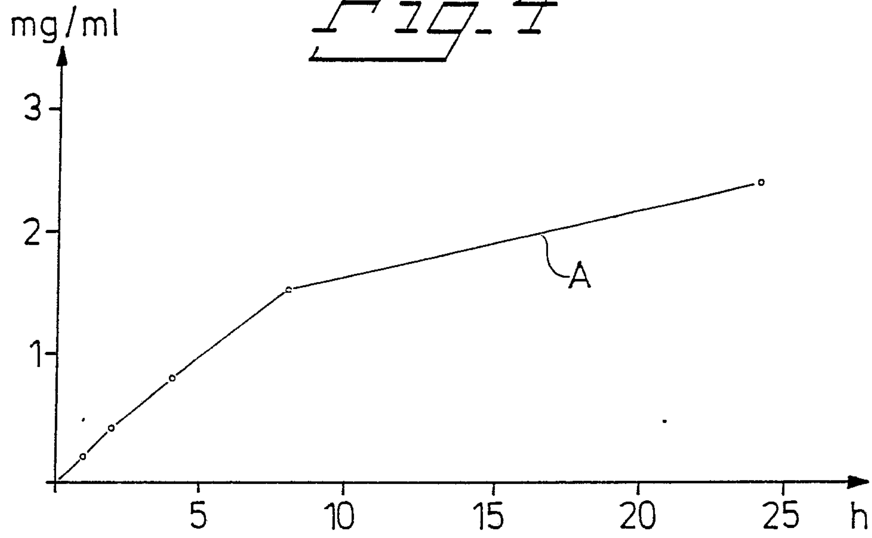

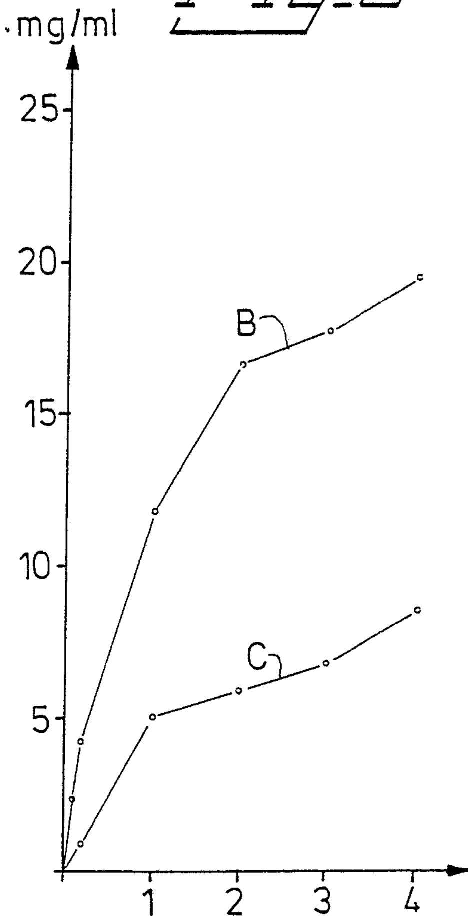

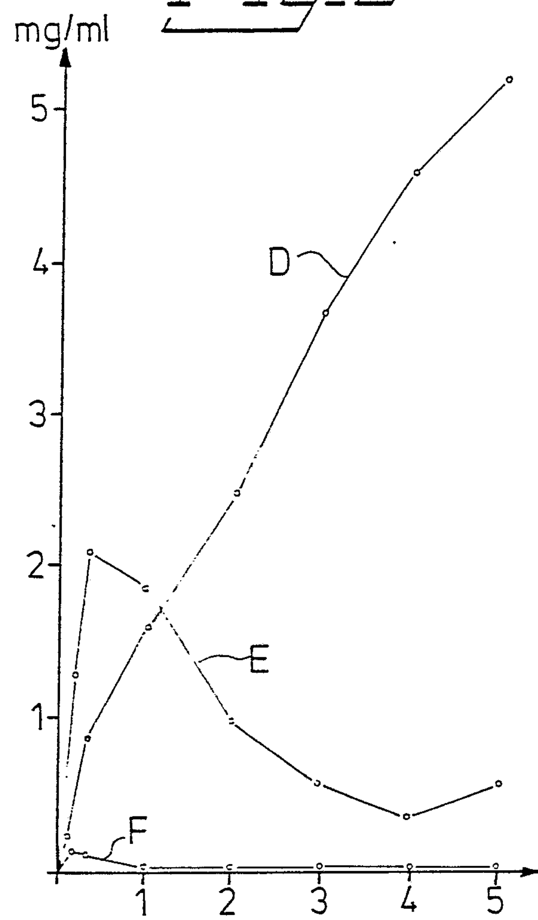

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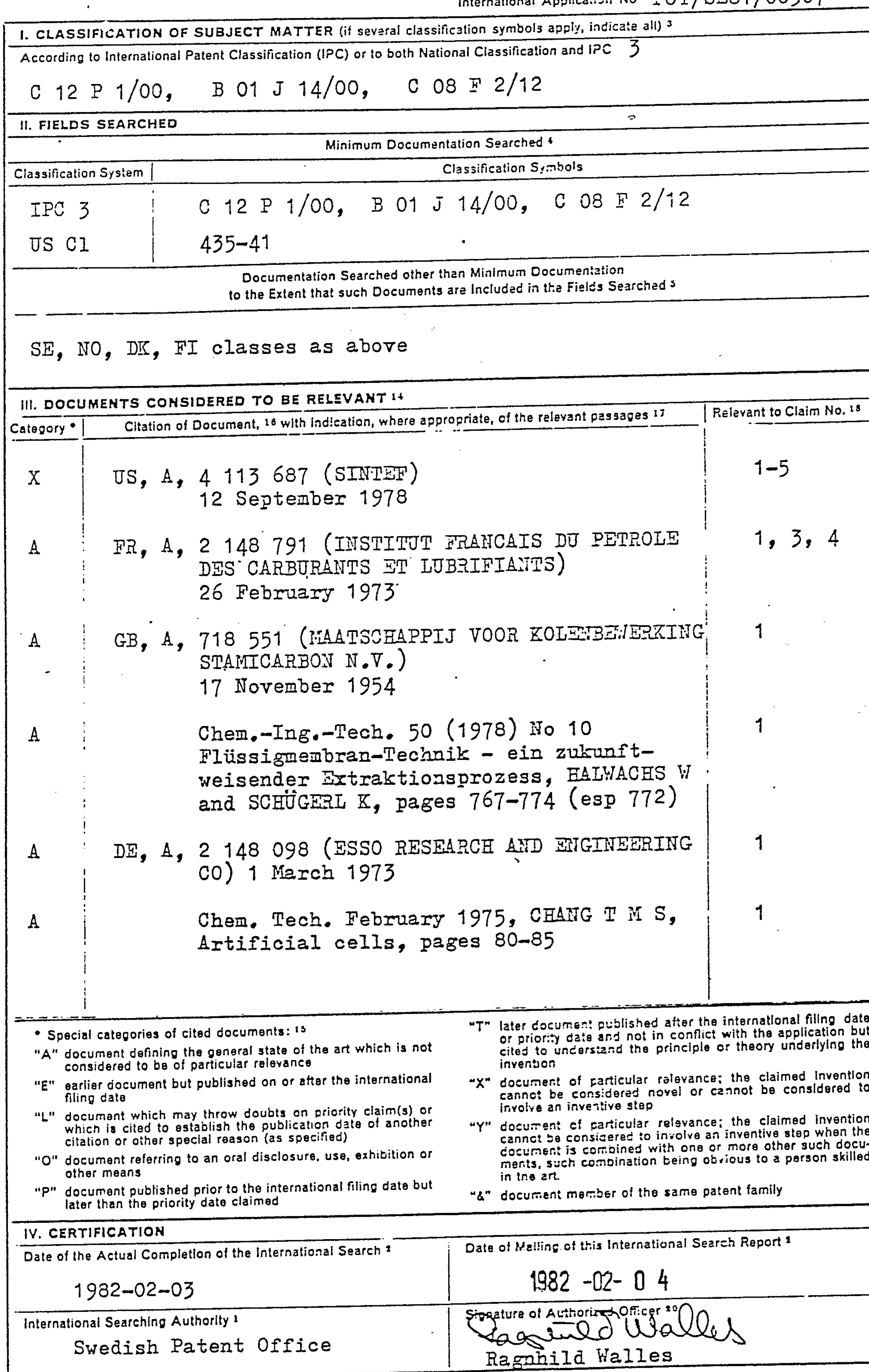

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE81/00307

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC 3

C 12 P 1/00, B 01 J 14/00, C 08 F 2/12

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
IPC 3	C 12 P 1/00, B 01 J 14/00, C 08 F 2/12
US Cl	435-41

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	US, A, 4 113 687 (SINTEF) 12 September 1978	1-5
A	FR, A, 2 148 791 (INSTITUT FRANCAIS DU PETROLE DES CARBURANTS ET LUBRIFIANTS) 26 February 1973	1, 3, 4
A	GB, A, 718 551 (MAATSCHAPPIJ VOOR KOLENBEWERKING STAMICARBON N.V.) 17 November 1954	1
A	Chem.-Ing.-Tech. 50 (1978) No 10 Flüssigmembran-Technik - ein zukunft-weisender Extraktionsprozess, HALWACHS W and SCHÜGERL K, pages 767-774 (esp 772)	1
A	DE, A, 2 148 098 (ESSO RESEARCH AND ENGINEERING CO) 1 March 1973	1
A	Chem. Tech. February 1975, CHANG T M S, Artificial cells, pages 80-85	1

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
1982-02-03	1982-02-04
International Searching Authority [1]	Signature of Authorized Officer [20]
Swedish Patent Office	Ragnhild Walles

Form PCT/ISA/210 (second sheet) (October 1981)

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