(54) Title: PROCESS FOR MODULATING THE CONCENTRATION OF DISSOLVED GASES IN LIQUID MEDIA

(57) Abstract

A process is provided for modulating the concentration of dissolved gases in liquid media, especially liquid media which are associated with biological materials e.g. aqueous culture media for cells, and waste products such as polluted water and sewage which are characterised by the presence of microorganisms. The process utilises a liquid transfer medium to effect the desired modulation and selecting physical properties of the liquid transfer medium and the manner in which the transfer medium is manipulated to facilitate the transfer of gases. The transfer medium (132) is transferred between zones (112, 114) operated at different temperatures in which the transfer medium exists respectively in the liquid and gaseous states, gas dissolves in the transfer medium in a zone (114) where the transfer medium is in the liquid state and separation of gas from the transfer medium takes place in a separate zone (112).
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PROCESS FOR MODULATING THE CONCENTRATION
OF DISSOLVED GASES IN LIQUID MEDIA

This invention relates to processes for modulating the concentration of dissolved gases in liquid media, especially liquid media which are associated with biological materials e.g. aqueous culture media for cells, and waste products such as polluted water and sewage which are characterised by the presence of microorganisms.

Many biochemical, chemical and physical processes require normally gaseous substances to be dissolved in liquid media. Similarly many processes exist in which it is necessary to remove dissolved gases from liquid media. In practice it is often difficult to modulate the concentration of dissolved gaseous components in a controlled manner. Thus for example the rate at which gases can be dissolved can depend upon factors which are difficult to control, for example bubble size and residence time, agitation conditions and properties of the liquid medium such as surface tension and viscosity.

The present invention overcomes such problems by utilising a liquid transfer medium to effect the desired modulation and selecting physical properties of the liquid transfer medium and the manner in which the transfer medium is manipulated to facilitate the transfer of gases.
In more specific aspects, this invention relates to processes for culturing cells in an aqueous growth medium. The invention particularly relates to procedures wherein an organic liquid which is immiscible with water is used for promoting transfer of gas to or from the growth medium.

The expression "process of culturing cells" as used herein is intended to encompass any procedure wherein prokaryotic or eukaryotic cells are grown or maintained in vitro. Such procedures include the growth, or maintenance under steady state conditions, of unicellular organisms or organisms in which the cells grow in the form of mycelia. They also include the growth, or maintenance under steady state conditions, of cells derived from multicellular organisms. Furthermore, the procedures encompassed by the term "process of culturing cells" include procedures carried out under both aerobic and anaerobic conditions suitable for maintaining cells in a viable state as well as procedures where cells enter the retardation or stationary phase, become senescent and even eventually die.

The processes of the invention thus includes procedures wherein cells are grown under aerobic or anaerobic conditions wherein cell multiplication occurs, with cell numbers and/or cell mass increasing. The process also includes procedures where cells are maintained under conditions where substantially no increase in cell numbers and/or mass occurs. The latter procedures include processes wherein cells are being cultured for the purpose of producing products of primary or secondary metabolism.

When cells are cultured under aerobic conditions, it is normally necessary to ensure that oxygen is transferred to the culture medium at a rate which is sufficiently high to maintain the required metabolic rate. Usually this is achieved by contacting the culture medium with an oxygen-containing gas, for example air, and adopting measures which encourage the efficient transfer of oxygen from the oxygen-containing gas to the culture medium.
Thus, for example, it is customary to introduce the oxygen containing gas into the culture vessel in the form of fine bubbles introduced through a sparger located at the bottom of the vessel. The high surface area of the bubbles ensures a high rate of oxygen transfer to the culture medium. Additional measures include the use of agitation, for example by providing agitators, impellors and associated structures such as baffles within the culture vessel in order to enhance the contacting of the culture medium with the oxygen containing gas. Other measures include the use of shaken vessels and in the case of cultured animal cells, the use of roller bottles in which a moving thin film of culture medium is continuously renewed.

Achieving a high rate of gas transfer is also important in circumstances where it is desired to remove gaseous products of metabolism from culture media. In the case of processes operated aerobically, such products of metabolism would normally include carbon dioxide. Other gaseous products of anaerobic metabolism include hydrogen and hydrogen sulfide. Thus when operating under anaerobic conditions, especially in the case of microorganisms which produce gaseous by-products such as hydrogen and hydrogen sulphide as products of anaerobic metabolism, it is customary to use a stream of inert gas in order to scrub these by-products from the culture medium.

Recently organic liquids which are immiscible with aqueous growth media have been investigated as possible candidates for promoting the transfer of gases to and from culture media. Thus, for example, perfluorochemicals (PFCs) have been extensively studied as gas exchange media. PFCs have been developed which are capable of dissolving large volumes of oxygen and carbon dioxide and their use as "blood substitutes" is now increasing (see e.g. Lowe, 1987). Similarly there has been considerable interest and research in the application of PFCs as oxygen carriers in both animal and microbial cell cultures (see King et al, 1989).
However the use of organic liquids as gas transfer media has hitherto found little or no practical exploitation despite the potential offered by the useful properties of such liquids. Thus, for example, PFCs have been used to transfer oxygen to aqueous culture media by spraying oxygenated PFC liquids into or onto cell cultures. The droplets of oxygenated PFC, being dense, settle to the bottom of the fermentor vessel, transferring oxygen to the medium as they fall. The PFC accumulates at the bottom of the vessel from where it can be recovered, reoxygenated and pumped back into the top as a spray, thus continuing the cycle. However the coarse droplet size and the relatively short residence time of the droplets in situ, severely limits the rate and efficiency with which oxygen can be transferred to the culture medium.

To avoid these disadvantages, attempts have been made to use PFCs in fermentation systems in the form of finely dispersed, surfactant-stabilised microemulsions in order to achieve efficient exchange gases between the PFCs and the aqueous growth media. However the use of PFCs in the form of microemulsions gives rise to a number of problems in particular:

i) physically handling PFC emulsions in fermentors can be problematical,

ii) recovering the valuable PFCs after the fermentation has finished can give rise to difficulties, mainly as a result of problems associated with separating the organic and aqueous phases (often requiring centrifugation of the stabilised emulsion),

iii) certain components of the fluid PFC-containing mixture can exert toxic effects on the cell population (specifically the surfactants required to maintain emulsion stability), and

iv) the large quantities of PFCs needed to be continuously added throughout the fermentation add to the expense of the procedures.
We have now developed a novel technique whereby organic fluids, in particular PFCs, can be used to modulate the concentration of dissolved gases in liquid media. This technique has widespread applicability, but it is especially useful for effecting highly efficient exchange of gases in both aerobic and anaerobic fermentation procedures, while essentially avoiding the problems associated with prior art procedures, and allowing increased yields of cell growth. In the novel procedure described herein, the organic fluid used to promote gas exchange can be continuously recycled and, of great importance to the user, easily recovered at the end of fermentation.

According to one aspect of the present invention, there is provided a process for modulating the concentration of a dissolved gas in a liquid medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that the organic fluid is transferred between zones operated at different temperatures in which the fluid exists respectively in the liquid and the gaseous states, gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state and separation of gas from the organic fluid takes place in a separate zone.

For example, separation may take place in a zone where the organic fluid is converted from the liquid to the gaseous state, in which case the gas may be evolved as the organic fluid passes from the liquid to the gaseous state.

The invention may more specifically be defined in terms of a process for transferring gas to or from a liquid medium, which comprises contacting the medium with a transfer fluid which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that the transfer fluid is transferred between zones operated at different temperatures in which the transfer fluid exists respectively in the liquid and the gaseous states, gas dissolves in the transfer fluid in a zone where the transfer fluid is in the liquid state and separation of gas from the transfer fluid takes place in a separate zone.
Thus according to a preferred aspect of the present invention, there is provided a process for modulating the concentration of a dissolved gas in a liquid medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that the organic compound is transferred between zones operated at different temperatures in which the compound exists respectively in the liquid and the gaseous states, whereby gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state, and is evolved when the organic fluid passes from the liquid to the gaseous state.

Alternatively separation of gas from the organic fluid may take place in a zone where organic fluid containing dissolved gas is contacted with the liquid medium. In this embodiment, gas may be transferred directly from the organic fluid to the liquid medium, or more preferably, the organic fluid is converted from the liquid to the gaseous state as it contacts the liquid medium and the evolved gas is then made available for dissolution in the liquid medium.

Thus in this manner of operation the invention further provides a process of for modulating the concentration of a dissolved gas in a liquid medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that the organic compound is transferred between zones operated at different temperatures in which the compound exists respectively in the liquid and the gaseous states, whereby gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state, and the organic fluid undergoes a change of state between the liquid and gaseous states while in contact with the liquid medium.

More specifically, in carrying out the method of the invention, organic fluid in the liquid state and containing dissolved gas (for example oxygen) may be contacted with the liquid medium, and dissolved gas transferred to the liquid medium without the organic liquid being converted to the gaseous state.
However, advantageously, during this contacting the organic fluid is converted to the gaseous state, dissolved gas is evolved and the evolved gas is dissolved in the liquid medium. To achieve this it would normally be necessary for the liquid medium to be at a temperature above the boiling point of the organic fluid. In this mode of operation, the organic fluid in the liquid state may be contacted with the liquid medium, the organic fluid converted to the gaseous state while in contact with the liquid medium and organic fluid in the gaseous state and liquid medium then separated from one another.

The evolved organic compound in the gaseous state is preferably condensed, then used to dissolve a further quantity of gas and recycled to release dissolved gas to the liquid medium.

Thus in this mode of operation there is provided in accordance with the invention a process for modulating the concentration of a dissolved gas in a liquid medium wherein transfer of gas (in particular oxygen) to the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that:

(i) the organic compound in the liquid state is contacted with the gas in a first zone operated at a temperature below the boiling point of the organic compound to form a solution of the gas in the organic liquid,
(ii) solution from step (i) is transferred to a second zone operated at a temperature above the boiling point of the organic liquid wherein the organic fluid passes from the liquid to the gaseous state with evolution of gas,
(iii) gas evolved in step (ii) contacts the liquid medium and dissolves therein,
(iv) organic fluid in the gaseous state from step (ii) is separated from the liquid medium,
(v) separated organic fluid in the gaseous state from step (iv) is condensed, and
(v) organic fluid in the liquid state from step (iv) is utilised to dissolve gas in step (i).
In an alternative method of operation according to the invention which is especially appropriate for operation wherein it is desired to reduce the concentration of a gaseous material dissolved in the liquid medium, organic fluid in the liquid state is contacted with the liquid medium and dissolved gas contained in the liquid growth medium is transferred to the organic liquid. The organic liquid containing the dissolved gas may then be separated from the liquid medium and if desired, recycled for reuse after stripping dissolved gas therefrom.

In this method of operation, organic fluid in the gaseous state may be contacted with the liquid medium, the organic fluid converted to the liquid state while in contact with the liquid medium and organic fluid in the liquid state and liquid medium may then be separated from one another.

Thus, organic fluid in the liquid state and containing dissolved gas may be separated from the liquid medium and transferred to a separate zone where the organic fluid is converted to the gaseous state, and dissolved gas is evolved and separated from the organic fluid. In this embodiment, the liquid medium would be at a temperature below the boiling point of the organic fluid.

More specifically, there is provided a process for modulating the concentration of a dissolved gas in a liquid medium wherein removal of gas from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that:

(1) the organic compound in the liquid state is contacted with the liquid medium in a first zone operated at a temperature below the boiling point of the organic compound whereby gas dissolved in the liquid medium is transferred to the organic liquid, to form a solution of the gas in the organic liquid,
(ii) solution from step (i) is transferred to a second zone operated at a temperature above the boiling point of the organic liquid wherein the organic fluid passes from the liquid to the gaseous state with evolution of gas,

(iii) gas evolved in step (iii) is separated from the organic fluid,

(iv) organic fluid in the gaseous state from step (ii) is condensed, and

(v) organic fluid in the liquid state from step (iv) is in step (i).

The process of the invention is especially applicable to modulating the concentration of gases in aqueous media, especially aqueous media which are intended for use as aqueous growth media for biological materials.

Thus, for example the process of the invention may be used to modulate the concentration of dissolved gases in a step of one of the following biological procedures:

(i) growth of prokaryotic or eukaryotic cells,

(ii) maintaining prokaryotic or eukaryotic cells under culture conditions leading to the production of a desired metabolite,

(iii) maintaining prokaryotic or eukaryotic cells under culture conditions leading to the elimination of an undesired substance,

(iv) maintaining prokaryotic or eukaryotic cells under culture conditions leading to conversion of a substance added to a culture medium to a chemically modified form,

(v) maintaining a mixed population of prokaryotic and/or eukaryotic cells under culture conditions leading to selective survival, proliferation, inhibition or death of one or more members of the population,

(vi) degradation of waste materials,

(vii) treatment of waste materials,

(viii) sequestration of heavy metals.
In its more specific aspects, when directed to the culturing of cells, the present invention provides a process of culturing cells in an aqueous growth medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the aqueous growth medium and is capable of dissolving the gas, characterised in that the organic fluid is transferred between zones operated at different temperatures in which the fluid exists respectively in the liquid and the gaseous states, gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state and separation of gas from the organic fluid takes place in a separate zone.

For example, separation may take place in a zone where the organic fluid is converted from the liquid to the gaseous state, in which case the gas may be evolved as the organic fluid passes from the liquid to the gaseous state.

Thus according to a preferred aspect of the present invention, there is provided a process of culturing cells in an aqueous growth medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the aqueous growth medium and is capable of dissolving the gas, characterised in that the organic compound is transferred between zones operated at different temperatures in which the compound exists respectively in the liquid and the gaseous states, whereby gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state, and is evolved when the organic fluid passes from the liquid to the gaseous state.

Alternatively separation of gas from the organic fluid may take place in a zone where organic fluid containing dissolved gas is contacted with aqueous growth medium. In this embodiment, gas may be transferred directly from the organic fluid to the aqueous growth medium, or more preferably, the organic fluid is converted from the liquid to the gaseous state as it contacts the aqueous growth medium and the evolved gas is then made available for dissolution in the aqueous growth medium.
Thus in this manner of operation the invention further provides a process of culturing cells in an aqueous growth medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the aqueous growth medium and is capable of dissolving the gas, characterised in that the organic compound is transferred between zones operated at different temperatures in which the compound exists respectively in the liquid and the gaseous states, whereby gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state, and the organic fluid undergoes a change of state between the liquid and gaseous states while in contact with the aqueous growth medium.

More specifically, in carrying out the method of the invention, organic fluid in the liquid state and containing dissolved gas (for example oxygen) may be contacted with the aqueous growth medium, and dissolved gas transferred to the aqueous growth medium without the organic liquid being converted to the gaseous state.

However, advantageously, during this contacting the organic fluid is converted to the gaseous state, dissolved gas is evolved and the evolved gas is dissolved in the aqueous growth medium. To achieve this it would normally be necessary for the aqueous growth medium to be at a temperature above the boiling point of the organic fluid. In this mode of operation, the organic fluid in the liquid state may be contacted with the aqueous growth medium, the organic fluid converted to the gasous state while in contact with the aqueous growth medium and organic fluid in the gaseous state and aqueous growth medium then separated from one another.

The evolved organic compound in the gaseous state is preferably condensed, then used to dissolve a further quantity of gas and recycled to release dissolved gas to the aqueous growth medium.
Thus in this mode of operation there is provided in accordance with the invention a process of culturing cells in an aqueous growth medium wherein transfer of gas (in particular oxygen) to the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the aqueous growth medium and is capable of dissolving the gas, characterised in that:

(i) the organic compound in the liquid state is contacted with the gas in a first zone operated at a temperature below the boiling point of the organic compound to form a solution of the gas in the organic liquid,
(ii) solution from step (i) is transferred to a second zone operated at a temperature above the boiling point of the organic liquid wherein the organic fluid passes from the liquid to the gaseous state with evolution of gas,
(iii) gas evolved in step (ii) contacts the aqueous growth medium and dissolves therein,
(iv) organic fluid in the gaseous state from step (ii) is separated from the aqueous growth medium,
(v) separated organic fluid in the gaseous state from step (iv) is condensed, and
(v) organic fluid in the liquid state from step (iv) is utilised to dissolve gas in step (i).

In an alternative method of operation according to the invention which is especially appropriate for anaerobic operation, organic fluid in the liquid state is contacted with the aqueous growth medium and dissolved gas contained in the aqueous growth medium is transferred to the organic liquid. The organic liquid containing the dissolved gas may then be separated from the aqueous growth medium and if desired, recycled for reuse after stripping dissolved gas therefrom.
In this method of operation, organic fluid in the gaseous state may be contacted with the aqueous growth medium, the organic fluid converted to the liquid state while in contact with the aqueous growth medium and organic fluid in the liquid state and aqueous growth medium may then be separated from one another.

Thus, organic fluid in the liquid state and containing dissolved gas may be separated from the aqueous growth medium and transferred to a separate zone where the organic fluid is converted to the gaseous state, and dissolved gas is evolved and separated from the organic fluid. In this embodiment, the aqueous growth medium would be at a temperature below the boiling point of the organic fluid.

More specifically, there is provided a process of culturing cells in an aqueous growth medium wherein removal of gas from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the aqueous growth medium and is capable of dissolving the gas, characterised in that:

(i) the organic compound in the liquid state is contacted with the aqueous growth medium in a first zone operated at a temperature below the boiling point of the organic compound whereby gas dissolved in the aqueous growth medium is transferred to the organic liquid, to form a solution of the gas in the organic liquid,
(ii) solution from step (i) is transferred to a second zone operated at a temperature above the boiling point of the organic liquid wherein the organic fluid passes from the liquid to the gaseous state with evolution of gas,
(iii) gas evolved in step (iii) is separated from the organic fluid,
(iv) organic fluid in the gaseous state from step (ii) is condensed, and
(v) organic fluid in the liquid state from step (iv) is in step (i).
The organic fluid used in the method of the invention may be a pure compound or a mixture of compounds.

Most preferably a PFC or mixture of PFCs is used. As used herein, the term PFC is used as an abbreviation for "perfluorochemical". It thus includes, but is not restricted to perfluorocarbons i.e. compounds of the formulae:

\[
\begin{align*}
\text{C}_n \text{F}_{2n+2} \\
\text{C}_n \text{F}_{2n} \\
\text{C}_n \text{F}_{2n-2} \\
\text{C}_n \text{F}_{2n-4} \\
\ldots \\
\text{C}_n \text{F}_{2n-x} \\
\end{align*}
\]

wherein \( n \) is a positive integer from 1 to 30, preferably from 4 to 20. In the most general of the above formulae (Formula I), \( x \) may be minus 2 (as in the first formula, zero (as in the second formula) or a positive even integer from 2 to 20, preferably from 2 to 15 and most preferably from 2 to 10.

The term "PFC" further includes compounds containing atoms in addition to carbon or fluorine, including atoms of nitrogen, oxygen and sulfur.

Such compounds can be represented by the general formulae

\[
\begin{align*}
\text{C}_n \text{N}_m \text{F}_{(2n-x+m)} & \quad \text{(II)} \\
\text{C}_n \text{O}_m \text{F}_{(2n-x)} & \quad \text{(III)} \\
\text{C}_n \text{S}_m \text{F}_{(2n-x)} & \quad \text{(IV)}
\end{align*}
\]

wherein \( n \) and \( x \) are as defined above and \( m \) is from 1 to 6, preferably from 1 to 3 and most preferably 1.
Specific examples include perfluoroalkanes, perfluorocycloalkanes and perfluoroalkyltetrahydrofurans.

Further examples of PFCs falling within categories I, II and III are described in Chapter 2 entitled "Fluorocarbon Fluids for use in the Electronics Industry" by D.S.L. Slinn and S.W. Green from the book entitled Preparations, Properties and Industrial Applications of Organicfluorine compounds" (Ed R.E. Banks, 1982, Ellis Horwood).

These include

perfluoropentanes \((\text{C}_5\text{F}_{12})\);  
perfluorohexanes \((\text{C}_6\text{F}_{14})\);  
perfluoro(methylcyclohexane) \((\text{C}_7\text{F}_{14})\);  
perfluoro(methyl/dimethylcyclohexane) \((\text{C}_7\text{F}_{14}/\text{C}_8\text{F}_{16})\);  
perfluoro(decalin/methyldecalin) \((\text{cis- and trans-} \text{C}_{10}\text{F}_{18})\); and  
perfluoro(decalin/methyldecalin) \((\text{cis- and trans-} \text{C}_{10}\text{F}_{18}/\text{C}_{11}\text{F}_{20})\).

Also described are fully-fluorinated (that is, perfluorinated) alkanes, ethers and tertiary amines, perfluoro-(2-n-butyltetrahydrofuran) perfluoro(2-n-propyltetrahydropyran) and perfluorotri-n-butylamine as well as low-molecular weight perfluorinated polyethers.

These PFC fluids are available under the tradenames Flutec® from ISC Chemicals Ltd., Fluorinert® from 3M and Galden® from Montedison SpA.

Other examples include perfluorodecalin, perfluorotripropylamine and perfluorotributylamine.
The invention will now be described in more detail by way of example with particular to the accompanying drawings of which

Figure 1 illustrates apparatus according to the invention adapted for growth of cells under aerobic conditions, and

Figure 2 illustrates apparatus according to the invention adapted for growth of cells under anaerobic conditions.

Referring to Figure 1, fermentation system 110 consists of fermentation vessel 112 connected to a system for circulating PFCs.

The circulation system includes a condensor 114 connected via conduit 116 to the head space 118 of fermentation vessel 112. The upper portion of the condensor is provided with a cooling coil 120 for condensing PFC vapour entering the condensor via conduit 116. The lower part of the condensor is fitted with a contacting device 122 for effecting gas exchange between oxygen containing gas introduced via line 124, and the PFC liquid resulting from condensation of the PFC vapour.

Any conventional gas/liquid contacting device may be used for effecting gas exchange. For example an oxygen-containing gas, e.g. air, may be bubbled through PFC liquid in a single or multiple stage gas/liquid bubbler or spray chamber. In addition to dissolving oxygen in the PFC liquid, condensor 120 and the associated contacting device 122 can also be used for removing carbon dioxide from the off-gases leaving fermentor 112 via conduit 116.

The outlet of the condensor is connected via conduit 126, pump 128 and conduit 130 to the sump 132 of the fermentation vessel 112. A resevoir 134 connects into line 130 via metering valve 136 for introducing make-up quantities of PFC into the system.
The fermentation vessel 112 is fitted with customary internal fittings including impeller 138 and temperature control coils 140.

In use of the apparatus illustrated, a charge of suitably sterilised culture medium is introduced into the fermentation vessel and innoculated with a starter culture of the cells to be grown. The temperature of the culture medium is maintained at a temperature suitable for growth by circulating heat exchange medium through the heat exchange coils 140.

The circulatory system is charged with a selected PFC fluid which has a boiling point which is below the temperature of the aqueous growth medium in the fermentation vessel. The temperature of the condensor 114 and the associated apparatus downstream thereof (conduit 126, pump 128, metering valve 13 and line 130) are maintained at a temperature below the boiling point of the PFC fluid so as to maintain it in the liquid state at least until it enters or is about to enter the fermentation vessel 112. The organic fluid is saturated with oxygen in device 122 and introduced in the liquid state into the sump of fermentation vessel 112 via conduit 126 and pump 128.

The PFC boils in the sump of the fermentation vessel and the oxygen gas dissolved therein is evolved. Bubbles 142 of PFC in the gaseous state and bubbles of evolved oxygen gas rise through the fermentation vessel and the oxygen is dissolved in the aqueous growth medium.

Gaseous PFCs accumulate in the head space of the fermentor and are passed via conduit 116 to the condensor 114 where they are cooled and condensed to the liquid state.
Fermentation system 110 described above in connection with Figure 1 of the drawing is especially adapted for growing cells under aerobic conditions utilising a water immiscible organic fluid as a carrier gas for introducing oxygen into the fermentation vessel. Thus, for example, where fermentation vessel 112 is used for growing animal cells under aerobic conditions in an aerobic fermentation medium maintained at 37°C, the PFC fluid used to transport oxygen would be selected to have a boiling point below 37°C. Preferably the organic fluid would have a boiling point within the range of 20-37°C. Thus when introduced into the sump 132 of the fermentation vessel in the liquid state at a temperature of approximately 25°C, the temperature of the organic fluid would increase until it boiled within the fermentation vessel, releasing its charge of oxygen which would dissolve in the aqueous fermentation medium and become available to the animal cells being grown.

The process of the invention is additionally applicable to the growth of cells under anaerobic conditions, in which case the water immiscible organic fluid would normally be used to remove gaseous products of metabolism from the aqueous growth medium.

Apparatus adapted for anaerobic use will now be described with reference to Figure 2.

Referring to Figure 2, fermentation system 210 consists of fermentation vessel 212 connected to a system for circulating PFCs.

The circulation system includes a pump 228 connected via line 230 to the sump 232 of vessel 212 and leading via conduit 226 to an evaporator 214. A reservoir 234 connects into line 230 via metering valve 236 for introducing make-up quantities of PFC into the system.
The lower part of evaporator 214 is provided with a heating coil 220 for evaporating PFC introduced into the evaporator via line 226. The upper portion of the evaporator 214 is fitted with a contacting device 222 for scrubbing PFC vapour evaporated in the lower part of the evaporator with a scrubbing liquid introduced via line 224.

The outlet of the evaporator is connected via conduit 216, to the head space of the fermentation vessel 212.

The fermentation vessel 212 is fitted with customary internal fittings including impellor 238 and temperature control coils 240.

In use of the apparatus illustrated in Figure 2, a charge of suitably sterilised culture medium is introduced into the fermentation vessel and inoculated with a starter culture of the cells to be grown. The temperature of the culture medium is maintained at a temperature suitable for growth by circulating heat exchange medium through the heat exchange coils 240.

The circulatory system is charged with a selected PFC fluid which has a boiling point which is above the temperature of the aqueous growth medium in the fermentation vessel. The temperature of the evaporator 214 and line 216 are maintained at a temperature above the boiling point of the PFC fluid so as to maintain it in the gaseous state at least until it enters or is about to enter the fermentation vessel 212.

The organic fluid is evaporated in evaporator 214 and after being scrubbed in contacting device 222 the PFC vapours are led via conduit 216 to the head space of the fermentor. The PFC vapour condenses within the fermentor and droplets of condensed PFC fall downwardly within the fermentor as a "rain" 242 of condensed liquid PFC. The condensed PFC scrubs the aqueous growth medium, and gaseous products of metabolism dissolve in the condensed PFC. The PFC containing the dissolved products of metabolism accumulate in the sump 232 of the fermentation vessel, where they are transferred via conduit 230 and pump 238 to evaporator 232.
PFC vapours leaving evaporator 214 via conduit 216 are cooled and condensed to form PFC liquid which is used to scrub gaseous products of metabolism from the aqueous fermentation medium as described above. The condensation of PFC vapour either takes place outside of fermentation vessel 218 in a separator condensor (not shown) or the vapour can be lead directly into fermentation vessel 218 where its temperature is reduced to below its boiling point and condensation takes place.

The apparatus illustrated in Figure 2 can be used for growing thermophilic microorganisms under anaerobic conditions. Thus, for example, the apparatus in Figure 2 may be used to grow the extreme thermophile *Pyrococcus furiosus*.

*P. furiosus* is a strictly anaerobic heterotroph and grows by a fermentative-type metabolism. Both simple and complex carbohydrates can be utilised with the production of carbon dioxide and hydrogen. If elemental sulphur (S₀) is present in the growth medium as the electron acceptor, H₂S is produced in addition to CO₂ and H₂. Effective growth of such anaerobic heterotrophs depends upon the removal of gaseous products of metabolism, such as H₂ and H₂S from the culture medium.

When culturing such extreme thermophiles, the aqueous culture medium would normally be maintained at a high temperature, for example, approximately 90°C and hydrogen or hydrogen sulphide would tend to accumulate in the culture medium as a by-product of metabolism.

Utilisation of PFC liquids in accordance with the invention provides a number of advantageous features:

1. Non-cell-disrupting aeration of the culture can be improved by utilising the well established oxygen carrying capacity of PFC liquids.
2. Rate of transport of the O₂ to the culture medium can be increased by rapid boiling-off of the PFC liquid allowing rapid recycling of low volumes of PFC liquid with corresponding cost savings.

3. The previous requirements for the production of a population of specific sized PFC liquid/ water micro-emulsion (for optimal oxygen transport properties) utilising energy sources (e.g. sonication) can be removed.

In addition, the toxic effects of the surfactant chemicals required for the production of stable microemulsions (e.g. Pluronic F-68) can also be avoided, an important feature, particularly for animal cell growth.

4. Varying the rate of reintroduction of recondensed PFC liquid to the fermentor can give fine control over gas exchange rates (e.g. aeration) throughout the fermentation.

5. Recovery of the PFC liquid at the end of the fermentation is achieved without requiring complicated phase separators or centrifugation. The cost of PCF per fermentation depends on the volume fraction required to support growth but is not likely to exceed usual medium costs and the bulk of the PFC fluid can, in any case, be recovered for reuse.

6. Given the physical and chemical stability of the PFC liquids used, the whole system (fermentor and PFC liquid recondenser loop) can be pre-sterilised, either chemically or by steam.

It will also be appreciated that by selecting an appropriate PFC liquid having a boiling point which is appropriate to operation either in connection with the embodiment described with reference to Figure 1 or in connection with the embodiment described with reference...
to Figure 2, the technique of the invention can be applied to mesophilic cultures in a wide variety of applications including:

- accelerated/forced waste water and effluent (urban/farm slurry) treatment
- sludge digestion
- bio-fuels from biomass (high efficiency and safe collection of biogas, etc.

A further advantage of the use of PFCs in accordance with the invention results from the fact that PFC fluids exhibit extremely low heats of vaporisation and condensation, e.g. the latent heat of vaporisation (at b.p.) of a typical PFC (FC87 b.p. 30°C) is only 24 cals/g and for FC 77 is 20 cals/g (c.f. water at 540 cals/g).

Thus in a typical, application carried out in the apparatus depicted in Figure 1, using a 2001 animal cell fermentation vessel, and assuming a 10% (201) volume fraction of PFC, the energy required to condense 37°C PFC vapour to 20°C liquid is 2728kcal – equivalent to a net cooling of the vessel medium by PFC of only 1.68°C per PFC fluid volume cycle.

Similarly, in the thermophilic fermentation as carried out in the apparatus depicted in Figure 2, the vessel-heating effect of introducing FC 77 PFC vapour (b.p. 110°C) at 120°C and condensing it (at 10% volume fraction) down to a vessel running temp of, for instance, 90°C represents a vessel temp. rise of only 4°C (energy requirement of 6,559 kcal).

These temperature changes can be seen therefore to be only a small proportion of the thermal mass of the vessels and will be readily compensated for by the existing vessel heater/cooler systems.
The external PFC evaporation/boiling and condenser units required for operating the process of the invention are therefore unlikely to be expensive in capital or running cost and will be able to employ conventional refrigerant motor systems possibly only marginally larger than those found on commercial freezer units.

Additional advantages which arise in the case of animal cell production relate to the oxygen usage demands of animal cells.

Animal cell cultures, running in batch mode, start at 2-3 x $10^5$ cells/ml, doubling daily to 2 million/ml by day 4 and decline after day 7. Perfusion cultures operate at 50-200 x $10^6$ cells/ml and typically last 40-80 days.

The oxygen demand of most cells falls in the range 2-10 x $10^{12}$ gms $O_2$ per cell per hour (average of 6). (Monoclonal cell lines require less (30%) $O_2$ than other cell types). Thus a typical maximum $O_2$ demand per litre per hour is (taking 5.33 ml of $O_2 = 7.6$ mg) 8.4 mls $O_2$ per litre of culture per hour.

If it is assumed that PFC liquids carry 40% (v/v) of $O_2$, each litre of cell culture would be supported by 21 ml PFC liquid, assuming 100% efficient transfer of $O_2$ to the aqueous phase of the culture medium on boiling of the PFC liquid.

If an arbitrary value of 25% efficiency of $O_2$ transfer is adopted and a PFC liquid recycling rate of 1 hour is used, average PFC liquid volumes required to support 11 of culture would be approx. 100 ml/l (10%). Again, recycling rates are likely to be much shorter than 1 hour, reducing the PFC liquid support volumes considerably e.g. mls PFC/l culture.

In the case of thermophilic systems, many of the extremely thermophilic (growth optimum at 90°C) Archaebacteria are sulphate metabolisers and many require the provision of elemental sulphur in the growth medium which generally results in the formation of $H_2S$ which is both toxic and corrosive to equipment. Continuous removal of this gas from the culture medium is currently very difficult.
Another of the more widely studied species is *Pyrococcus furiosus* which has been studied for the production of a range of enzymes including proteases, amylases, pullulanase and α-glucosidase. All of the enzymes are of significant commercial importance and improving methods of obtaining them in commercially viable quantities from these bacteria is of great interest. The organism has an optimal growth temperature of 95°C and is able to grow in the absence of sulphur but it produces hydrogen which becomes toxic to the organism and limits growth yield and the resulting low cell yields render industrial scale-up of such fermentation commercially non-viable. The current optimum method for removal of hydrogen is to flush the vessel with an anaerobic (e.g. Argon) gas.

The opportunity to use PFC liquids to remove toxic and growth inhibitory by-products may considerably improve biomass yields which, at present, is the key limiting factor to the exploitation of these novel and potentially important microorganisms.

The method of the invention is not only applicable to the transfer to aqueous growth media of gases which play a major role in metabolism (e.g. oxygen in the case of aerobic fermentations), but it can also be used to establish or maintain any desired or necessary gas or gas mixture at an appropriate concentration within the medium (e.g. to exert fine control of microaerophilic conditions).

The invention may also be applied to the "harvesting" of gases produced by fermentations, e.g. "biogas", CH₄, H₂, NH₃, H₂S, SO₂, CO₂ etc. Other areas of application include photosynthetic cell growth.

By way of summary, the processes of the invention have a wide applicability in both the biological and non-biological fields.
Volatile PFC gas exchange capability is seen as being applicable to improving cellular
1. animal
2. microbial (including fungal and yeast)
3. plant,
and that they (PFC's) may be used to improve processes aimed at:
(a) producing cells themselves (biomass, cell paste, animal cell lines, etc.)
(b) producing the products of cell metabolism (secondary products e.g. alcohol(s), antibiotics, etc.)
(c) employing cells to effect the modification of a specific "material/substance" that they may use either as a substrate or may be modified by the growth of the cells in the presence of the "material". This term modification could then include:
degradation (of wastes),
treatment (of wastes),
sequestration (e.g. heavy metals)
breakdown (e.g. of recalcitrants ( pcb's, etc..)
assimilation
digestion

The PFC's could then be used to improve the process by either adding specific gases to aid the process of remove gases (of any type) that might otherwise inhibit the process.

The "material/substance" could then come from different sources:
(i) biological (e.g. sewage waste, foodstuffs (and wastes derived from foodstuff manufacture), etc.)
(ii) chemical, both
    inorganic (e.g. sequestration of (heavy) metals in biomining, metal containing wastes, nuclear materials, etc.)
    and
    organic (chemicals/polymers used in pharmaceuticals, petrochemicals, pesticide manufacture, dyestuffs, foodstuffs, xenobiotics, etc..)
1. A process for modulating the concentration of a dissolved gas in a liquid medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that the organic fluid is transferred between zones operated at different temperatures in which the fluid exists respectively in the liquid and the gaseous states, gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state and separation of gas from the organic fluid takes place in a separate zone.

2. A process according to Claim 1 wherein separation of gas from the organic fluid takes place in a zone where the organic fluid is converted from the liquid to the gaseous state.

3. A process according to Claim 2 wherein gas is evolved as the organic fluid passes from the liquid to the gaseous state.

4. A process for modulating the concentration of a dissolved gas in a liquid medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that the organic compound is transferred between zones operated at different temperatures in which the compound exists respectively in the liquid and the gaseous states, whereby gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state, and is evolved when the organic fluid passes from the liquid to the gaseous state.

5. A process according to any preceding claim wherein organic fluid in the liquid state and containing dissolved gas is contacted with the liquid medium and dissolved gas is transferred to the liquid medium.
6. A process according to any preceding claim wherein organic fluid in the liquid state and containing dissolved gas is contacted with the liquid medium whereby the organic fluid is converted to the gaseous state, dissolved gas is evolved and evolved gas dissolved in the liquid medium.

7. A process according to any preceding claim wherein organic fluid in the liquid state is contacted with the liquid medium, the organic fluid is converted to the gaseous state while in contact with the liquid medium and organic fluid in the gaseous state and liquid medium are separated from one another.

8. A process according to any preceding claim wherein the liquid medium is at a temperature above the boiling point of the organic fluid.

9. A process for modulating the concentration of a dissolved gas in a liquid medium wherein transfer of gas to the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that:

(i) the organic compound in the liquid state is contacted with the gas in a first zone operated at a temperature below the boiling point of the organic compound to form a solution of the gas in the organic liquid,

(ii) solution from step (i) is transferred to a second zone operated at a temperature above the boiling point of the organic liquid wherein the organic fluid passes from the liquid to the gaseous state with evolution of gas,

(iii) gas evolved in step (ii) contacts the liquid medium and dissolves therein,

(iv) organic fluid in the gaseous state from step (ii) is separated from the liquid medium,

(v) separated organic fluid in the gaseous state from step (iv) is condensed, and

(v) organic fluid in the liquid state from step (iv) is utilised to dissolve gas in step (i).
10. A process according to any preceding claim wherein the gas is oxygen.

11. A process according to any of Claims 1 to 4 wherein organic fluid in the liquid state is contacted with liquid medium and dissolved gas contained in the liquid medium is transferred to the organic liquid.

12. A process according to Claim 11 wherein organic fluid in the gaseous state is contacted with liquid medium, the organic fluid is converted to the liquid state while in contact with the liquid medium and organic fluid in the liquid state and liquid medium are separated from one another.

13. A process according to Claim 11 or Claim 12 wherein organic fluid in the liquid state and containing dissolved gas is separated from the liquid medium and transferred to a separate zone where the organic fluid is converted to the gaseous state, and dissolved gas is evolved and separated from the organic fluid.

14. A process according to any of Claims 11 to 13 wherein liquid medium is at a temperature below the boiling point of the organic fluid.

15. A process for modulating the concentration of a dissolved gas in a liquid medium wherein removal of gas from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that:

(i) the organic compound in the liquid state is contacted with the liquid medium in a first zone operated at a temperature below the boiling point of the organic compound whereby gas dissolved in the liquid medium is transferred to the organic liquid, to form a solution of the gas in the organic liquid.
(ii) solution from step (i) is transferred to a second zone operated at a temperature above the boiling point of the organic liquid wherein the organic fluid passes from the liquid to the gaseous state with evolution of gas,
(iii) gas evolved in step (iii) is separated from the organic fluid,
(iv) organic fluid in the gaseous state from step (ii) is condensed, and
(v) organic fluid in the liquid state from step (iv) is contacted with liquid medium in step (i).

16. A process for modulating the concentration of a dissolved gas in a liquid medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the liquid medium and is capable of dissolving the gas, characterised in that the organic compound is transferred between zones operated at different temperatures in which the compound exists respectively in the liquid and the gaseous states, whereby gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state, and the organic fluid undergoes a change of state between the liquid and gaseous states while in contact with the liquid medium.

17. A process according to Claim 16 wherein the organic fluid is converted from the liquid to the gaseous state while in contact with the liquid medium.

18. A process according to Claim 16 wherein the organic fluid is converted from the gaseous to the liquid state while in contact with the liquid medium.

19. A process according to any preceding claim wherein the liquid medium is an aqueous medium.

20. A process according to Claim 19 wherein the liquid medium is an aqueous growth medium for a biological material.
21. A process according to any preceding claim wherein the modulation of the concentration of dissolved gas comprises a step of one of the following biological procedures:

(i) growth of prokaryotic or eukaryotic cells,
(ii) maintaining prokaryotic or eukaryotic cells under culture conditions leading to the production of a desired metabolite,
(iii) maintaining prokaryotic or eukaryotic cells under culture conditions leading to the elimination of an undesired substance,
(iv) maintaining prokaryotic or eukaryotic cells under culture conditions leading to conversion of a substance added to a culture medium to a chemically modified form,
(v) maintaining a mixed population of prokaryotic and/or eukaryotic cells under culture conditions leading to selective survival, proliferation, inhibition or death of one or more members of the population,
(vi) degradation of waste materials,
(vii) treatment of waste materials,
(viii) sequestration of heavy metals.

22. A process of culturing cells in an aqueous growth medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the aqueous growth medium and is capable of dissolving the gas, characterised in that the organic fluid is transferred between zones operated at different temperatures in which the fluid exists respectively in the liquid and the gaseous states, gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state and separation of gas from the organic fluid takes place in a separate zone.

23. A process according to Claim 22 wherein separation of gas from the organic fluid takes place in a zone where the organic fluid is converted from the liquid to the gaseous state.
24. A process according to Claim 23 wherein gas is evolved as the organic fluid passes from the liquid to the gaseous state.

25. A process of culturing cells in an aqueous growth medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the aqueous growth medium and is capable of dissolving the gas, characterised in that the organic compound is transferred between zones operated at different temperatures in which the compound exists respectively in the liquid and the gaseous states, whereby gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state, and is evolved when the organic fluid passes from the liquid to the gaseous state.

26. A process according to any of Claims 22 to 25 wherein organic fluid in the liquid state and containing dissolved gas is contacted with the aqueous growth medium and dissolved gas is transferred to the aqueous growth medium.

27. A process according to any of Claims 22 to 26 wherein organic fluid in the liquid state and containing dissolved gas is contacted with the aqueous growth medium whereby the organic fluid is converted to the gaseous state, dissolved gas is evolved and evolved gas dissolved in the aqueous growth medium.

28. A process according to any of Claims 22 to 27 wherein organic fluid in the liquid state is contacted with the aqueous growth medium, the organic fluid is converted to the gaseous state while in contact with the aqueous growth medium and organic fluid in the gaseous state and aqueous growth medium are separated from one another.

29. A process according to any of Claims 22 to 28 wherein aqueous growth medium is at a temperature above the boiling point of the organic fluid.
30. A process of culturing cells in an aqueous growth medium wherein transfer of gas to the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the aqueous growth medium and is capable of dissolving the gas, characterised in that:

(i) the organic compound in the liquid state is contacted with the gas in a first zone operated at a temperature below the boiling point of the organic compound to form a solution of the gas in the organic liquid,
(ii) solution from step (i) is transferred to a second zone operated at a temperature above the boiling point of the organic liquid wherein the organic fluid passes from the liquid to the gaseous state with evolution of gas,
(iii) gas evolved in step (ii) contacts the aqueous growth medium and dissolves therein,
(iv) organic fluid in the gaseous state from step (ii) is separated from the aqueous growth medium,
(v) separated organic fluid in the gaseous state from step (iv) is condensed, and
(v) organic fluid in the liquid state from step (iv) is utilised to dissolve gas in step (i).

31. A process according to any of Claims 22 to 30 wherein the gas is oxygen

32. A process according to any of Claims 22 to 25 wherein organic fluid in the liquid state is contacted with aqueous growth medium and dissolved gas contained in the aqueous growth medium is transferred to the organic liquid.

33. A process according to Claim 32 wherein organic fluid in the gaseous state is contacted with aqueous growth medium, the organic fluid is converted to the liquid state while in contact with the aqueous growth medium and organic fluid in the liquid state and aqueous growth medium are separated from one another.
34. A process according to Claim 32 or Claim 33 wherein organic fluid in the liquid state and containing dissolved gas is separated from the aqueous growth medium and transferred to a separate zone where the organic fluid is converted to the gaseous state, and dissolved gas is evolved and separated from the organic fluid.

35. A process according to any of Claims 32 to 34 wherein aqueous growth medium is at a temperature below the boiling point of the organic fluid.

36. A process of culturing cells in an aqueous growth medium wherein removal of gas from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the aqueous growth medium and is capable of dissolving the gas, characterised in that:

(i) the organic compound in the liquid state is contacted with the aqueous growth medium in a first zone operated at a temperature below the boiling point of the organic compound whereby gas dissolved in the aqueous growth medium is transferred to the organic liquid, to form a solution of the gas in the organic liquid,

(ii) solution from step (i) is transferred to a second zone operated at a temperature above the boiling point of the organic liquid wherein the organic fluid passes from the liquid to the gaseous state with evolution of gas,

(iii) gas evolved in step (iii) is separated from the organic fluid,

(iv) organic fluid in the gaseous state from step (ii) is condensed, and

(v) organic fluid in the liquid state from step (iv) is contacted with aqueous growth medium in step (i).
37. A process of culturing cells in an aqueous growth medium wherein transfer of gas to or from the medium is promoted by use of an organic fluid, which when in the liquid state is immiscible with the aqueous growth medium and is capable of dissolving the gas, characterised in that the organic compound is transferred between zones operated at different temperatures in which the compound exists respectively in the liquid and the gaseous states, whereby gas dissolves in the organic fluid in a zone where the organic fluid is in the liquid state, and the organic fluid undergoes a change of state between the liquid and gaseous states while in contact with the aqueous growth medium.

38. A process according to Claim 37 wherein the organic fluid is converted from the liquid to the gaseous state while in contact with the aqueous growth medium.

39. A process according to Claim 37 wherein the organic fluid is converted from the gaseous to the liquid state while in contact with the aqueous growth medium.

40. A process according to any preceding claim wherein the organic fluid comprises a perfluorochemical (PFC).

41. A process according to any preceding claim wherein the organic fluid comprises a perfluorochemical (PFC).

42. A process according to Claim 41 wherein the PFC is a perfluorocarbon having the formula

\[ C_n F_{2n-x} \]  

wherein n is a positive integer from 1 to 30, and x is minus 2, zero or a positive even integer from 2 to 20.
43. A process according to Claim 41 wherein the PFC is a compound represented by the general formulae

\[ C_{n+m}F^{(2n-x+m)} \quad (II) \]
\[ C_{n+m}O \quad (III) \]
\[ F^{(2n-x)} \quad (IV) \]

wherein wherein \( n \) is a positive integer from 1 to 30, \( x \) is minus 2, zero or a positive even integer from 2 to 20 and \( m \) is from 1 to 6.

44. A process according to Claim 41 wherein the PFC is a perfluoroalkane, a perfluorocycloalkane or a perfluoroalkylyttetrahydrofuran.

45. A process according to Claim 41 wherein the PFC is selected from the following (including mixtures thereof):

- perfluoropentanes \((C_5\text{F}_{12})\);
- perfluorohexanes \((C_6\text{F}_{14})\);
- perfluoro(methylcyclohexane) \((C_7\text{F}_{14})\);
- perfluoro(dimethylcyclohexane) \((C_8\text{F}_{16})\);
- perfluorodecalin \((c\text{cis- and/or trans-}C_{10}\text{F}_{18})\); and
- perfluoro(methyldecalin) \((c\text{cis- and/or trans-}C_{11}\text{F}_{20})\).

46. A process according to Claim 41 wherein the PFC is a perfluorinated alkane, ether or tertiary amine.

47. A process according to Claim 41 wherein the PFC is perfluoro(2-n-butyttetrahydrofuran), perfluoro(2-n-propylttetrahydropyran) or perfluorotri-n-butylamine.
Fig. 1.
Fig. 2.
INTERNATIONAL SEARCH REPORT

PCT/GB 93/01392

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12M/03 C12M/04 C12N5/00 C02F3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12M C12N C02F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>FR,A,2 177 051 (TANABE SEIYAKU CO., LTD.) 2 November 1973</td>
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Further documents are listed in the continuation of box C. Patent family members are listed in annex.

Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "P" document published prior to the international filing date but later than the priority date claimed

Date of the actual completion of the international search 29 October 1993

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Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl
Fax (+31-70) 340-3016

Authorized officer
BEVAN, S

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</tr>
</tbody>
</table>

Form PCT/ISA/210 (patent family annex) (July 1992)