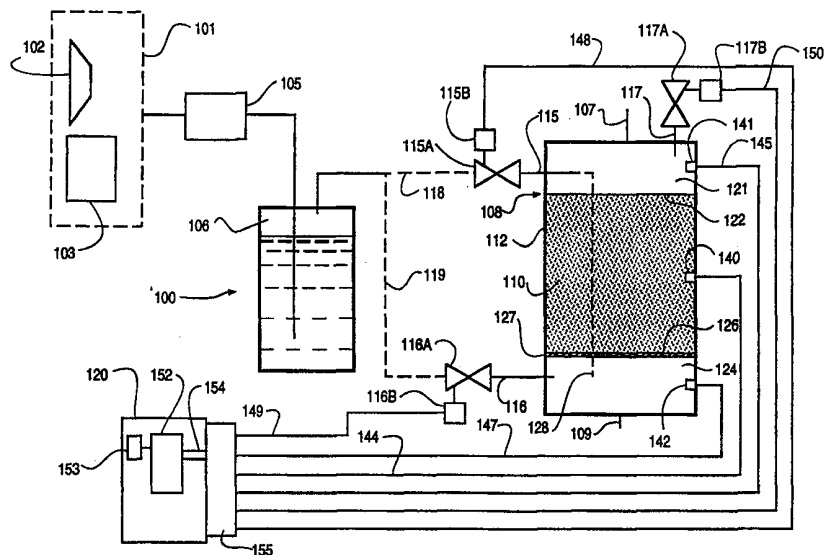


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(54) Title: CULTURING APPARATUS AND CULTIVATING METHOD



(57) Abstract

Disclosed is a culturing apparatus (100) that includes a gas-permeable solid phase culturing bed (110) disposed within an interior space of a vessel (108). The culturing bed comprises a solid substrate that includes a biological agent disposed thereon. A gas inlet (115, 116) permits selective introduction of gas into the vessel (108) under pressure sufficient to cause gas to flow through at least a portion of the bed (110). Also disclosed is a method of cultivating a biological agent. The method comprises providing a gas-permeable culturing bed, flowing a gas through the bed and recovering at least a portion of the biological agent. The gas preferably is an oxygen-containing gas, whereby the flow of gas through the bed provides convective mass transfer of oxygen to the biological agent. The apparatus and method of the invention permit "pressure pulsing" of gas through the bed.

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**CULTURING APPARATUS
AND CULTIVATING METHOD**

5

RELATED APPLICATION

This application claims priority to prior United States Provisional Patent Application Serial No. 60/082,332, filed April 20, 1998, the contents of which
10 are hereby incorporated by reference in their entireties.

TECHNICAL FIELD OF INVENTION

15

The present invention relates generally to the field of culturing biological agents. More specifically, the invention is directed towards a method of cultivating a biological agent and towards a culturing apparatus useful in connection with the cultivation of a biological agent.

20

BACKGROUND OF THE INVENTION

It is well known that certain biological products (such as enzymes, organic acids, vitamins, extracellular
25 proteins, amino acids, antibiotics, and the like) can be produced by biological agents, particularly by bacteria and other prokaryotic organisms such as yeasts, molds, and fungi, as well as eukaryotic species such as plant or animal cells. Many efforts have been made to increase
30 the quantities of desirable biological products that are produced by such biological agents by facilitating the growth of same. By way of example, during the Second World War, those skilled in the art sought to accelerate the production of penicillin in view of the large demand
35 for treating battle wounds. These efforts gave birth to the then new technology of submerged fermentation. This process involves submerging the biological agents (e.g.,

5 fungal mycelia in the production of penicillin) in a tank that contains a liquid medium that functions as a nutrient solution. The biological agents excrete or otherwise produce the desired biological product in the liquid culture medium. After a period of cultivation (often lasting several days), the liquid is filtered off, and the biological product is extracted therefrom. Other submerged liquid media cultivating methods are known in the art.

10 Techniques for growing biological agents in submerged liquid media, such as submerged fermentation, have not been fully satisfactory. For example, a significant problem, especially with aerobic processes, is that the liquid in which the biological agent is
15 immersed hinders the transfer of oxygen to the biological agents to be cultivated. In addition, use of the liquid media raises environmental concerns because of problems associated with the disposal thereof.

More recently, solid phase processes for cultivating
20 biological agents have been employed. For example, composting is a technique that involves aerobic bacterial decomposition of solid organic waste. Generally, growth of a biological agent, such as mycelia, on a solid substrate occurs more readily than when the biological
25 agent is submerged in liquid. As a result, biological agents grow more quickly when exposed to ambient air, as compared to when such agents are submerged in liquid. It is believed that biological agents that are cultivated on solid substrate surfaces absorb oxygen directly from the
30 ambient atmosphere. In processes that utilize a liquid medium for culturing the biological agents, the transfer of oxygen to the cells is thus relatively hindered. Accordingly, the use of solid phase processes enhances

growth and other metabolic activities as compared with liquid phase processes.

Despite the advantages associated with solid phase processes for culturing biological agents, these
5 processes have not met entirely with success. For example, the solid substrates in a solid phase process is usually packed to form a bed which acts as a good heat insulator. In many solid phase processes, such as in the degradation of grass clippings and leaves by composting,
10 heat is generated by the metabolic activities of the biological agents disposed within the solid phase. The heat, not being able to dissipate quickly, causes the temperature to increase inside the composting pile. This temperature increase helps to kill microorganisms and
15 insects, which is desired in the composting of yard waste. However, if the solid phase process is intended for high levels of digestion of the solid wastes and/or for the manufacture of biological products, the temperature increase undesirably may tend to terminate
20 the biological process prematurely.

Another problem suffered by solid phase processes relates to the delivery of oxygen into the porous beds, which delivery is often necessary for the growth of biological agents. Generally, at least a portion of the
25 biological agent in a bed will be disposed within the bed, where it is not exposed to ambient oxygen. As such, oxygen can only be supplied to the agent by slow molecular diffusion through the bed, which diffusion will occur more slowly than is often desired and which may be
30 rate limiting in the growth of the biological agent. Furthermore, solid substrates and the biological agents often form large aggregates which further impede the flow

of oxygen into the solid phase, thereby further reducing the supply of oxygen to the biological agent.

It is a general object of the invention to provide a culturing apparatus and method that permit culturing of biological agents such that desired biological products can be produced more readily than via conventional techniques. Another general object of the invention is to provide a culturing apparatus and method for delivering oxygen to a biological agent within a culturing bed.

THE INVENTION

It has now been found that biological agents can be cultivated in a culturing apparatus which includes a gas-permeable bed disposed within a vessel and which provides for convective flow of gas through the bed. The convective flow of gas through the bed provides for relatively enhanced heat and/or mass transfer to and from the bed as compared with conventional culturing processes. This enhanced heat and/or mass transfer has been found to enhance the growth of biological agents within the bed.

In accordance with one embodiment of the invention, the vessel is provided with a gas inlet that permits the introduction of gas into the vessel. The gas-permeable bed contains a biological agent and a substrate suitable for growing the biological agent. The gas is introduced through the gas inlet into the vessel under pressure sufficient to cause gas to flow through at least a portion of the bed. Desirably, the bed includes a gas outlet, and both the gas inlet and gas outlet are fluidically coupled to valves operable between a closed

state and at least one open state. An operator may open and close the valves to thereby modify the flow of gas through the vessel and to thereby moderate the temperature of the bed within a desired temperature
5 range. Most preferably, the valves are operated so as to operate the vessel in a "pressure pulsing" mode, wherein gas flow through the apparatus is cyclically varied.

In accordance with another aspect of the present invention, a method of cultivating a biological agent is
10 provided. The method includes the step of providing a gas-permeable culturing bed that includes a substrate that is biologically conducive for culturing of a biological agent, flowing a gas through the bed so as to cause the gas to come into convective thermal contact
15 with at least a portion of the biological agent and/or to cause convective mass transfer with the biological agent, and recovering at least a portion of the biological agent. The biological agent preferably has an aerobic activity, and the gas preferably is an oxygen-containing
20 gas.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic representation of a culturing
25 apparatus in accordance with the invention;

Fig. 2 is a graphical representation of the variance of the pressure within the vessel of the apparatus shown in Fig. 1 with time, when operated in a pressure pulsing
30 mode in accordance with a preferred mode of the invention;

Fig. 3 is a representational illustration of gas flow through the culturing bed;

Fig. 4 is a flow diagram illustrating a possible control logic for operation of a gas outlet valve in the culturing apparatus shown in Fig. 1;

5

Fig. 5 is a flow diagram illustrating a possible control logic for operation of a gas inlet valve in the culturing apparatus shown in Fig. 1;

10 Fig. 6 is a flow diagram illustrating another possible control logic for operation of a gas outlet valve in the culturing apparatus shown in Fig. 1;

15 Fig. 7 is a flow diagram illustrating another possible control logic for operation of a gas inlet valve in the culturing apparatus shown in Fig. 1;

20 Fig. 8 is a flow diagram illustrating another possible control logic for operation of a gas outlet valve in the culturing apparatus shown in Fig. 1;

25 Fig. 9 is a flow diagram illustrating another possible control logic operation of a gas inlet valve in the culturing apparatus shown in Fig. 1;

Fig. 10 is a flow diagram illustrating another possible control logic for operation of a gas outlet valve in the culturing apparatus shown in Fig. 1;

30 Fig. 11 is a flow diagram illustrating another possible control logic for operation of a gas inlet valve in the culturing apparatus shown in Fig. 1;

Fig. 12 is a flow diagram illustrating another possible control logic for operation of a gas outlet valve in the culturing apparatus shown in Fig. 1;

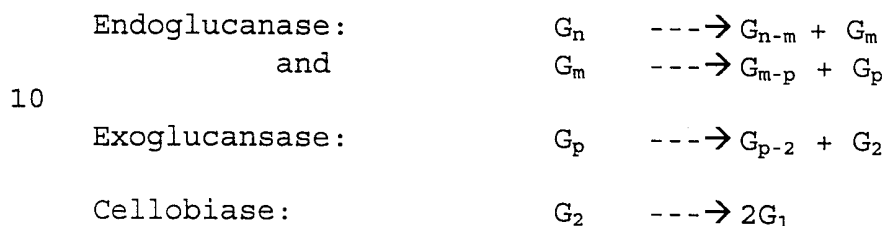
- 5 Fig. 13 is a flow diagram illustrating another possible control logic for operation of a gas inlet valve in the culturing apparatus shown in Fig. 1.

DESCRIPTION OF PREFERRED EMBODIMENTS

The invention generally is directed towards the culturing (i.e., cultivating) of any biological agent that can be grown on a substrate, and thus, for example, the invention is contemplated to find utility in connection with both aerobic and anaerobic culturing of biological agents, such as by fermentation. By "culturing" and "cultivating" are contemplated an increase in the amount of the biological agent as a result of growth of the biological agent on or in conjunction with the substrate. It is contemplated that a biological agent may be desirably cultivated for its ability to produce a biological product (e.g., the product penicillin is obtained from mycelial cells). "Culturing" (and "cultivating") of the biological agent thus are also intended to encompass obtaining a desired biological product directly, as well as obtaining the biological agent itself. Examples of biological agents that may be cultured and recovered in accordance with the invention include bacteria, yeasts, spores, fungi, molds, plant and animal cells, and generally any biological agent as may be known or as hereinafter may be discovered. The agent preferably is an aerobic agent, but also may be an agent that has an anaerobic activity. The biological products that may be recovered include enzymes, amino acids, vitamins, organic acids, extracellular proteins, antibodies, and, in general, any biologically produced material.

For example, the invention may be employed in connection with the cultivation of cellulase-producing biological agents, which cultivation preferably is accomplished on a cellulosic substrate. The cellulose-

hydrolyzing cellulases include three enzymes,
 endoglucanase (EC 3.2.1.74, commonly known as C_x),
 exoglucanase (EC 3.2.1.91, also known as C_1), and
 cellobiase (EC 3.2.1.21). These enzymes work together to
 5 convert cellulose into glucose. The activities of these
 enzymes are summarized below:



15 wherein G represents an anhydroglucose unit, and can
 range from about 2,000 to 10,000 in cellulose (G_n thus
 representing cellulose); and wherein n, m, and p are
 integers wherein $n > m > p$. In this series of reactions,
 endoglucanase cleaves the cellulose molecule into two
 20 anhydroglucose chains; exoglucanase cleaves cellobiose
 (G_2 , composed of two anhydroglucose units) from a linear
 anhydroglucose chain; and cellobiase converts the
 cellobiose to glucose. Among many cellulase-producing
 microbes, those belonging to *Trichoderma* are known for
 25 their high productivity of mixtures of all three
 cellulase components. *Aspergillus niger* also is a
 producer of cellulase enzymes, although some strains of
A. niger are known to be producers of cellobiase only.
 The invention is not limited to cultivation of the
 30 foregoing biological agents, but instead is contemplated
 to be generally applicable to the cultivation of any
 suitable biological agent.

The invention generally contemplates both a
 culturing apparatus and a method for cultivating a
 35 biological agent. The apparatus is generally shown in

Figs. 1. With reference thereto, the apparatus 100 includes a source 101 of gas, which source may be, for example, an air compressor 102, an oxygen tank 103, or any other suitable gas source. When the biological agent
5 has an aerobic activity, the gas preferably is an oxygen containing gas, such as purified oxygen or air, but it is further contemplated that a different gas may be used in connection with the invention in some embodiments. For example, it may be desired periodically to flush the
10 vessel with an inert gas, such as nitrogen gas. In such case, the source 101 may include a tank of compressed nitrogen (not shown). The gas optionally but preferably is filtered through a filter 105 and is humidified at humidifier 106, either of which may, if desired, be
15 provided with a heating and/or cooling mechanism, such as a coiled jacket containing heating and/or cooling coils (not shown). After leaving the humidifier 106, the air passes into a culturing vessel 108 that includes a gas-permeable culturing bed 110 disposed within an interior
20 space defined by a wall 112 thereof.

The culturing bed 110 comprises a substrate that includes a biological agent disposed thereon, the substrate being biologically conducive for the growth of the biological agent. The substrate preferably is
25 selected for compatibility with the biological agent to be cultured, and thus, for example, when cellulase enzymes are being cultured, the substrate may comprise recycled paper fibers, wood chips, or other cellulose-containing source. Other substrate suitable for use in
30 conjunction with the invention includes grains, such as wheat bran, cracked corn, whole grain rice, and other organic materials such as soluble proteins. More generally, any substrate that provides physical and

nutritive support for the biological agent and that is suitably porous so as to be gas permeable may be employed. By "gas permeable" is contemplated that the bed allows gas to flow therethrough in convective thermal communication with at least a portion of the biological agent in the bed and/or in convective mass transfer communication with at least a portion of the biological agent. The substrate may comprise an inorganic material (such as a diatomaceous earth) in admixture with a nutritive substrate such as urea, grain, or other suitable nutrient. The selection of a particular substrate for a given biological agent to be cultivated is contemplated to be within the level of ordinary skill in the art.

The substrate is provided in the form of a solid phase substrate, by which is contemplated a porous or gas-permeable substrate that preferably is wet (i.e., has sufficient moisture to promote the growth of the biological agent), but that is not submerged in a liquid bath. Preferably, the substrate is initially provided in the form of wet discrete plural packings, the substrate packings being packed in the bed with sufficient void volume to allow the bed to be permeable to at least compressed gas. It is contemplated that the substrate packings will form a friable cohesive mass after the biological agent has been allowed to grow for a sufficient length of time, and thus no longer may be identifiable as discrete packings.

The vessel 108 shown in Fig. 1 is equipped with two gas inlets 115, 116, each having a gas inlet valve 115A, 116A, controlled by a respective valve actuator 115B, 116B. The vessel also is equipped with a gas outlet 117 and a gas outlet valve 117A which is operated by a gas

outlet valve actuator 117B. The valve actuators further may be controlled by a controller 120, as discussed in more detail hereinbelow. The controller, valves and accompanying valve actuators are deemed optional in
5 connection with the invention, and thus the vessel may be equipped with none, one, two or all three of the illustrated valves 115A, 116A, and 117A, or may be equipped with further valves and valve actuators if desired.

10 The gas passes into the vessel 108 via one or both of gas inlets 115, 116. The gas inlet or inlets preferably are charged with water vapor prior to introducing gas to thereby avoid drying out of the culturing bed. In the illustrated embodiment, gas
15 leaving the humidifier 106, passes along either or both of paths 118, 119 through respectively valves 115A and 116A and gas inlets 115 and 116 (the paths 118, 119 being shown in broken lines as optional alternatives). The vessel 108 preferably includes a head space 121 proximal
20 a boundary 122 of the bed 110 and a bottom space 124 proximal another boundary 126 of the bed 110. For example, the bed 110 may rest on a screen 127 within the vessel 108, the screen 127 permitting fluid flow therethrough but not permitting solid contents of the bed
25 110 to pass into the bottom space 124. Entering gas may pass either into the head space 121 via gas inlet 115, or the bottom space 124 via gas inlet 116 (or via optional path 128 via gas inlet 115, path 128 being shown in broken lines as an optional alternative).

30 In operation, gas is introduced to the vessel in a manner such that gas flows through at least a portion of the bed 110. It is contemplated in preferred embodiments of the invention that the gas will flow through the

entirety of the bed to thereby maximize the benefits attainable in accordance with the invention. It is contemplated that the gas may not be able to flow through the entirety of the bed, however, such as when the bed contains occlusions or when the gas inlet and outlet are positioned to cause gas flow only through a portion of the bed. As shown in Fig. 3, the bed 110 comprises discrete plural particles 130 of substrate with a biological agent disposed thereon. Gas flows through a first boundary 131 of the bed, as represented by arrow 132, through at least a portion 133 of the bed, as represented by arrow 134, and through a second boundary 135 of the bed, as represented by arrow 136. The first and second boundaries 131 and 135 are preferably but not necessarily coextensive with the boundaries 122, 126 between the bed 110 and the head space 121 and bottom space 124 respectively (as shown in Fig. 1). For example, gas may be introduced into the bottom space 124 of the vessel 108 through gas inlet 116 under a pressure greater than ambient pressure. The gas will flow from the bottom space 124 through the bed 110 into the head space 121 and out the gas outlet 117. In another embodiment, the vessel is pressurized by introducing gas at the head space through gas inlet 115 with the gas outlet valve 117A being closed. Gas will flow into the bed 110, even if the vessel is not equipped with a bottom space. The gas outlet valve 117A then may be opened to allow gas to escape from the vessel 108.

The biological agent is disposed on the bed, i.e., on a surface of the bed or within the bed. Preferably, the biological agent is homogeneously dispersed throughout the bed. While it is not intended to limit the invention to a particular theory of operation, it is

believed that the passage of gas through the culturing bed will enhance mass and/or heat transfer within the bed as a result of convective transfer of oxygen through the bed. For example, in the case of aerobic growth of a biological agent, the biological agent disposed within or growing into interior portions of the bed will be allowed to breathe more readily than if gas were not allowed to flow through the bed as a result of convective transfer of oxygen through the bed. It is further believed that volatile metabolites will be allowed to escape from the bed via convective mass transfer.

The passage of gas through the bed may also affect heat transfer and temperature within the bed. For example, in the case of an oxygen-containing gas, increasing the flow of oxygen to the bed may cause the temperature within the bed to increase or decrease. It is believed that the increase in oxygen flow rate will cause the metabolic activity of microorganisms within the bed to increase (thus tending to increase the temperature within the bed) but also increasing the convection of heat away from the bed (thus tending to decrease the temperature within the bed, so long as the gas is at a temperature lower than that of the bed). The rate at which the bed temperature is caused to increase as a result of microorganism activity may be more than, less than, or equal to, the rate at which the temperature is caused to decrease as a result of convection of heat away from the bed. Thus, depending on the stage of fermentation, the flow rate of the gas, and other factors, the temperature within the bed may be caused to increase or to decrease by increasing the oxygen flow rate. The effect of the flow rate preferably is empirically determined for a given apparatus and process.

In the case of anaerobic agents within the bed, it is contemplated that an increase in flow rate will not cause an increase in the metabolic activity within the bed. In such case, increasing the flow rate of gas will be
5 expected to cause the bed temperature to decrease if the gas is at a lower temperature than the bed, and to increase if the gas is at a higher temperature.

If desired, heat may be removed from the bed by flowing an inert gas (such as nitrogen) through the bed.
10 Alternatively, non-humidified gas, or gas that has a low humidity, may be introduced to thereby cause evaporation of water vapor from the bed and to thereby remove latent heat from the bed. For example, the gas inlet may not be charged with water vapor prior to introducing gas into
15 the bed, thus causing evaporative cooling of the bed. Alternatively, the gas may be cooled prior to entering the bed, or the vessel may be equipped with cooling coils (not shown).

In a particularly preferred embodiment, the
20 apparatus is operated in a "pressure pulsing" mode. By "pressure pulsing" in one embodiment is contemplated cyclically pressurizing and depressurizing the vessel. An example of the pressure profile within the vessel generated in accordance with such a pressure pulsing is
25 shown in Fig. 2 (pressure being given as gauge pressure). "Pressure pulsing" also encompasses cyclical increasing and subsequently decreasing the cyclical flow rate of gas through the bed. In either case, by "cyclical" is contemplated repeating the pressurization/depressuriza-
30 tion or increase in flow rate/decrease in flow rate operations at least once, and more preferably, at least five times, after the initial pair of operations is completed. In general, the pair of operations may be

repeated as many times as desirable for a given application.

It will be apparent to those skilled in the art that the pressure pulsing may be accomplished using various
5 embodiments of the apparatus shown in Fig. 1, as well as in any other suitable manner. For example, the vessel may be equipped with a gas inlet 115 with no gas inlet valve, and a gas outlet with gas outlet valve 117A. Pressure pulsing then may be accomplished by closing the
10 gas outlet valve 117A, allowing the pressure within the vessel to build and preferably to hold when the pressure within the vessel reaches that pressure of the gas incoming through the gas inlet 115, subsequently opening the gas outlet valve to thereby allow the pressure within
15 the vessel to decrease to ambient pressure, and repeating this operation. Alternatively, gas may enter the vessel through gas inlet 116, which may or may not be equipped with a gas inlet valve, or may come in through gas inlet 115 via optional path 128. In another embodiment of the
20 invention, gas enters the vessel through both gas inlets 115 and 116, at least gas inlet 116 of which is equipped with a gas inlet valve 116A. Pressure pulsation may be accomplished by periodically opening and closing gas inlet valve 116A to allow respectively greater and
25 smaller amounts of gas to enter the vessel 108.

The gas inlet and outlet valves 115A, 116A and 117A may be manually operated. In a preferred embodiment of the invention, each valve is equipped with a valve actuator 115B, 116B, and 117B respectively, each of which
30 modifies the state of its respective valve (for example, by fully opening or fully closing each valve or by incrementally increasing or decreasing the amount of fluid that may flow through said valve). The valves and

valve actuators may be integral with the vessel or may be remote from the vessel. For example, the valve may be associated with the gas source (such as the valve on a pressure vessel), or may be associated with the

5 compressor. The valve actuator may be, for example, a switch for actuating the compressor (the compressor thus serving as a valve). Alternatively, the valve actuator may be, for example, a solenoid actuator, the gas valve thus comprising a solenoid valve. Alternatively, the

10 valve and actuator may be any other suitable devices. The valve actuators may be controlled by a controller 120.

In a highly preferred embodiment of the invention, the apparatus is equipped with a temperature sensor 140

15 measuring the temperature of the bed, and/or one or more pressure sensors 141, 142, measuring a pressure within the vessel 108 (it being contemplated that the pressure reported in the head space by pressure sensor 141 may differ from that reported in the bottom space by pressure

20 sensor 142). In accordance with this embodiment of the invention, the controller 120 communicates with the temperature sensor 140 via line 144, and communicates with the one or more pressure sensors via lines 145, 147. The controller further communicates with valve actuators

25 115B, 116B, 117B via lines 148, 149, and 150 respectively.

The controller may be any electronically or otherwise operated mechanism. In some cases, the controller may comprise simple control logic circuitry,

30 such as a wired circuit, or may comprise a timer. In one embodiment, the controller comprises a microprocessor or microcontroller 152 including a timer 153, a data bus 154, and an I/O interface 155 via which the sensors and

valve actuators communicate with the microprocessor or microcontroller 152. The sensors provide signals to the controller to thereby communicate temperature or pressure data to the microprocessor or microcontroller 152, and
5 the microprocessor sends control signals to the valve actuators 115B, 116B, and 117B for modifying the state of the gas inlet and/or outlet valves.

The microprocessor or microcontroller or the logic circuitry may be programmed via any suitable manner for
10 accomplishing pressure pulsation. For example, if the pressure pulsation is accomplished with a microcontroller or microprocessor, via opening and closing the gas outlet valve, one suitable control program is diagrammatically illustrated in Fig. 4. At step 160, a delay register in
15 the microprocessor or microcontroller is reset with a closed reference time, i.e., the length of time that the gas output valve should remain closed. After the timer has indicated the passage of this amount of time, the microprocessor or microcontroller, at step 161, sends an
20 open valve signal to the gas outlet valve actuator. At step 162, the delay register is reset with an open reference time, i.e., a value indicating the amount of time that the gas outlet valve should remain open. Subsequently, after such time has passed, a close valve
25 signal is sent to the gas outlet valve actuator at step 163, and this cycle is repeated. The open reference time and closed reference times preferably are empirically determined for the given apparatus and method, and may take into account the amount of time required for the
30 valve actuator to accomplish respectively opening and closing of the valve. It is further contemplated that an operator may terminate the control loop at any time desired. If pressure pulsation is to be accomplished via

cyclical opening and closing of a gas inlet valve, the program diagrammatically shown in Fig. 5 is one appropriate program for the microprocessor or microcontroller, steps 164-167 corresponding
5 substantially to steps 160-163 shown in Fig. 4. It should be understood that the control programs shown in Figs. 4 and 5 and in the subsequent figures, while illustrated as control programs for a microcontroller or microprocessor, may be implemented by logic circuitry or
10 by other suitable control mechanisms.

Upon growth of the biological agent, the temperature within the bed may increase as the rate of metabolic activity within the bed increases. The heat may cause premature termination of the growth of the biological
15 agent, for example, if the temperature reaches an undesirably high level. On the other hand, if the temperature decreases below the desired operating range of the enzyme, the metabolic activity within the bed undesirably may decrease. When the bed includes a
20 temperature sensor, the microprocessor or microcontroller may be programmed in accordance with the control logic shown diagrammatically in Fig. 6 with respect to the control of a gas outlet valve. At step 170 a reference temperature, or desired maximum operating temperature of
25 the vessel is obtained, for example, by receiving user input or a stored memory variable. At step 171, the microprocessor or microcontroller receives a signal from the temperature sensor, and at step 172, this temperature received is evaluated as against the reference
30 temperature. If the received temperature is not yet as great as the reference temperature, after a delay 173 control passes to step 171. If, on the other hand, the temperature within the bed has reached or exceeded the

reference temperature, a signal is sent to the gas outlet valve actuator at step 174 to thereby cause the gas outlet valve to open and to thereby cause depressurization of the vessels. After a delay 175
5 another signal is sent to the gas valve actuator at step 176 to thereby cause the gas valve to close. Control passes to step 171 after another delay 177. It is contemplated that the delays at steps 173, 175, and 177 and the reference temperature may be empirically
10 determined for a given apparatus and biological agent. The reference temperature preferably defines or is below the maximum temperature within the desired activity range of the biological agent.

In an alternative embodiment, as shown in Fig. 7,
15 the microprocessor or microcontroller may be used to control a gas inlet valve actuator. The program is comparable to that shown in Fig. 6 except that a close valve signal is sent at step 178 and an open valve signal is sent at step 179.

20 A further alternative program is shown in Fig. 8. In this embodiment, a gas outlet valve is caused to open when the temperature within the bed has reached a first reference temperature, and the gas outlet valve is caused to close when the temperature within the bed has fallen
25 to a second reference temperature. The first and second reference temperatures are obtained as step 180, such as by receiving input from a user or by retrieving data valves from memory storage. Steps 181-185 are comparable to steps 171-175 respectively of the embodiment shown in
30 Fig. 6. After the valve has been opened and after the delay 185, a signal is again received from the temperature sensor at step 186. At step 187, this temperature is compared to the second reference

temperature to determine whether the temperature within the bed has fallen to or below the second reference temperature. If not, after a delay 188 control passes to step 186. If, on the other hand, the temperature has
5 fallen to or below the second reference temperature, control passes to step 189, where a close valve signal is sent to the gas outlet valve actuator. After a delay 190, control passes to step 180. The first and second reference temperatures preferably define or are within
10 the range of the upper and lower temperatures of the desired activity range of the biological agent. The bed temperature should remain between about 30° C and 42° C for both *Trichoderma* and *A-niger*. Fig. 9 illustrates a similar embodiment wherein the microprocessor or
15 microcontroller is used to control a gas inlet valve actuator. In this embodiment, a close valve signal is sent at step 191 and an open valve signal is sent at step 192.

As an alternative to measuring the temperature
20 within the bed and opening or closing valves in response thereto, the valve may include one or more pressure sensors 141, 142. Most preferably, the vessel includes one pressure sensor, which preferably is located in the head space when the gas inlet is in the bottom space of
25 the vessel and is preferably located in the bottom space of the vessel when the gas inlet is in the head space of the vessel. Figs. 10-13 are comparable to Figs. 6-9, respectively and diagrammatically illustrate the programming of the microprocessor or microcontroller
30 whereby the gas inlet or outlet valve may be opened or closed in response to pressure changes within the vessel. Steps 170'-192' are comparable to steps 170-192 in Figs 6-9. Fig. 10 illustrates operation of a gas outlet

valve, in which an open valve signal is given (at step 174') when the pressure has reached at least a reference pressure, and a close valve signal is subsequently given (at step 177') after a delay 176'. Fig. 11 is comparable
5 to Fig. 10 and shows the control of a gas inlet valve, wherein an open valve signal is given at step 178' and a close valve signal at step 179'.

Fig. 12 illustrates operation of a gas outlet valve wherein an open valve signal is given (at step 184') when
10 the pressure has reached at least a first reference pressure and a close valve signal (at step 189') when the pressure has fallen to or below a second reference pressure. Fig. 13 is comparable to Fig. 12 in the operation of a gas inlet valve, wherein a close valve
15 signal is given at step 191' and an open valve signal at step 192'. The pressures and delays in the foregoing programs may be empirically determined for a given apparatus or biological agent.

The foregoing description of programs that may be
20 used in conjunction with pressure pulsing is by no means meant to be exhaustive, and it is contemplated that those skilled in the art may find many other ways to program, a microprocessor or microcontroller or to operate an apparatus in accordance with the invention. For example,
25 instead of closing or opening a valve completely, it is contemplated that a suitable program might cause the valve actuator to incrementally open or incrementally close a valve, as may be appropriate. It is further contemplated that other microprocessor or microcontroller
30 programs or other logical circuitry or control scheme may be developed by those skilled in the art.

After growth of the biological agent, at least a portion of the biological agent is recovered from the

vessel. By "recovering" the biological agent is contemplated any action by which any portion of the biological agent, or the biological product produced by the biological agent, is removed from the vessel, whether
5 such biological agent or product was present initially in the vessel or was grown in the vessel. For example, as shown in Fig. 1, the vessel 108 may be provided with a liquid intake port 107 and a drain 109. To recover the biological agent, sterilized water may be fed into the
10 vessel via intake port 107, and the biological agent may be recovered via removal of the liquid through the drain 109. The biological agent then may be continue to be cultivated. Water or nutrients may be added as may be appropriate.

15 The following non-limiting Examples are provided for illustration of the present invention.

EXAMPLE 1

20 A two liter New Brunswick glass jar fermentor was equipped with a metal mesh screen to hold a wet porous solid bed having a depth of about 10 centimeters. The fermentor was equipped with a gas inlet for introducing
25 air to the bottom space of the fermentor (beneath the screen), a gas outlet valve leading from the head space (above the bed), a gas outlet solenoid valve (Omega Technologies Co.) and an electrical timer (ChronTrol) serving as a controller. Air was allowed to flow into
30 the bottom space and then upwards through the porous bed into the head space. Pressure pulsation was created by periodically opening and closing the gas outlet valve. The bed included a substrate with an *Aspergillus niger* culture disposed thereon.

With pressure pulsation, the bacteria growth was uniform and heavy throughout the bed. The porous bed turned totally black because of the heavy formation of the black colored spores by the *A. niger* culture. The whole bed was "loose" and, in fact, it was difficult to take the whole bed out of the jar without having portions of the bed falling off.

Example 1 was repeated without pressure pulsation. Only the bottom portion of the porous bed near the air inlet became blackened, and the central portion of the bed became tightly packed, with little mycelial growth and even less spore formation.

EXAMPLE 2

In a set-up identical to that in EXAMPLE 1, sterile water was pumped into the jar from the bottom up to extract enzyme from the Porous Bed. After the liquid extract was pumped out, there were still some liquid which slowly drained from the bed into the bottom space. Once the drainage was complete, air flow was re-introduced to start the bioprocess again. Once good growth was observed again, the extraction was repeated. The first extraction was done three days after the inoculation. The second extraction was done 24 hours later, and the extraction was then repeated daily for five days. The whole residual mass was then taken out of the jar and thoroughly extracted with added detergent.

This method of enzyme production was done with the *A. niger* culture for cellobiase and also with the *Trichoderma* culture for the whole cellulase complex. The results were as follows:

Cellulases by <i>Trichoderma</i>		Cellobiase by <i>A. niger</i>	
Washing/Day	FPU Extracted	Washing/Day	IU Extracted
1 st /3 rd	14149.8	1 st /3 rd	17049.6
2 nd /4 th	12929.7	2 nd /4 th	17290.
3 rd /5 th	9855.2	3 rd /5 th	17406.9
4 th /6 th	6645.8	4 th /6 th	15222.2
Final/8 th	9802.	Final/7 th	37294.2
Wash with Surfactant Drainage	16560. 831.		
Total	70773.5		104,263

From these results and the measured amounts of the solid enzyme products, the enzyme productivity was calculated to be 806 FPU/hour-liter for cellulase complex from

5 *Trichoderma*. In the case of cellobiase-producing *A. niger*, a productivity of 620 International Units/hour-liter was achieved. One international "filter paper unit" (FPU) is defined to be the amount of cellulases that can produce one micro-mole of glucose per minute

10 from cellulose.

COMPARATIVE EXAMPLE 1

A *Trichoderma* culture was cultivated on recycled paper fibers in a solid phase fermentation in a 1000 ml fermentor (a laboratory Erlenmeyer flask) to produce high potency cellulases. The fermentation was accomplished by placing the flask at room temperature on a laboratory bench top. After the fermentation was completed, the whole wet solids were air dried to become the final enzyme product. This product contains 246 FPU/gram, from which the productivity of the fermentor can be calculated to be 234 FPU/hour-liter of fermentor volume. The productivity of the solid phase fermentation was found to be higher than those of submerged fermentation of different *Trichoderma* cultures, reported in the literature and collected in the following Table:

Prior Art Cellulase Productivity
in Liquid Phase Fermentation

Culture	Substrate	Productivity in FPU/hour-liter
RUT-30	5% Solkfloc	87
RUT 30	10% steam exploded aspen	83
RUT 30	2.2% steam exploded poplar	37
RL-P37	6% lactose	158
SVG-17	3.7% pretreated grain husk	60

It is thus seen that the productivity of the method used in Example 2 for fermentation of the *Trichoderma* culture is more than 500% better than the productivity of liquid submerged fermentation reported in the prior art, and 344% higher than that of Comparative Example 1.

EXAMPLE 3
and
COMPARATIVE EXAMPLES 2-5

5 A mixture including 200 g corn fiber (14% moisture content, from A.E. Staley, Decatur, IL), 60 g ground corn, 30 ml corn steeping liquid (A.E. Staley), 4.0 g $(\text{NH}_4)_2\text{SO}_4$, 2.0 g K_4PO_4 , and 600 ml water was prepared (final moisture content was about 75%). This mixture was
10 autoclaved for 30 minutes at 121° C and allowed to cool to form a substrate. To this substrate was added 20 g solid A. *Niger* culture in a septic hood.

EXAMPLE 3

15 Most (80%) of the substrate/biological agent mixture was transferred to a 2 L fermentor with a 15 cm packing bed height equipped with a gas inlet, a gas outlet, and a gas outlet solenoid valve. The fermentator was operated
20 with the pressure pulse profile shown in Fig. 2. An air inlet was provided for the bottom space, and air was allowed to flow continuously into the bed. The gas outlet led from the head space in the vessel, and the gas outlet valve was controlled by a timer.

25 Fermentation was allowed to proceed for 60 hours, after which the substrate became black. Subsequently, 100 ml water containing 0.2% $(\text{NH}_4)_2\text{SO}_4$ was introduced to wash out enzymes. This washing out was repeated once each day for three days. Samples of the extract were
30 analyzed each day for enzyme activity. Before activity analysis, 10 g was solid sample were mixed with 10 ml 0.05N pH 4.5 citrate buffer in a 250 ml flask. The mixture was shaken for three hours in a 25° shaker, filtered to remove solids and spores, and stored in a
35 refrigerator.

COMPARATIVE EXAMPLES 2-5

The substrate mixture prepared as discussed above
5 was divided and transferred to four 250 ml Erlenmeyer
flasks as follows:

	Comparative Example 2	30 g
	Comparative Example 3	60 g
	Comparative Example 4	40 g (with 100 ml water)
10	Comparative Example 5	40 g (with 100 ml 5% glucose solution).

The mixture of Comparative Examples 2, 4, and 5 were set in a 30° C shaker at 200 rpm to begin fermentation. The mixture of Comparative Example 3 was allowed to ferment at ambient temperature without shaking.

The following results were obtained:

Physical Phenomena

Day	Example 3		Comparative Example 2		Comparative Example 3	
	Color	Temp.	Color	Temp.	Color	Temp.
0	Brown	25°C	Brown	25°C	Brown	25°C
1	Light White	35°C	Brown	30°C	Brown	25°C
2	Grey	33°C	Brown	30°C	Brown	25°C
3	Black	33°C	Light White	30°C	Brown	25°C
4	Black	32°C	Light White	30°C	Light White	25°C
5	Black	30°C	White	30°C	White	25°C
6	Black	28°C	White	30°C	White	25°C
7	Black	28°C	Grey	30°C	White	25°C

Enzyme Activity (glucoamylase)

	Example 3	Comparative Example 2	Comparative Example 3	Comparative Example 4	Comparative Example 5
IU/g	522.5	213.3	157.4	97.29	153.25
IU/ml	-	-	-	8.27	13.02
IU/mg Protein	2.12	1.52	1.12	0.86	1.15

It is thus seen that the enzyme activity per mg
 5 protein realized in accordance with Example 3 was
 superior as compared with that realized in accordance
 with the Comparative Examples. The following further
 results were obtained in connection with Example 3:

10

Production of Glucoamylase
 During Solid State fermentation

Sample	Volume (ml)	IU/ml	Total IU	IU/g, dry	%	Hour
1 st Wash	1000	12.29	12290	63.03	12.06	60
2 nd Wash	1050	10.23	10741.5	55.08	10.54	84
3 rd Wash	1060	13.03	13811.8	70.83	13.56	108
Final solid	14885.5	4.37	65049.6	333.59	63.84	132
Total	-	-	101892.9	522.5	100	132

Final solid dry weight: 195 grams
 15 Moisture content of final solid culture: 86.9%
 Volume: 14885.5 ml

Production of Cellobiase During
Solid State Fermentation

Sample	Volume (ml)	IU/ml	Total IU	IU/g, dry	%	Hour
1 st Wash	1000	14.07	14070	72.15	11.61	60
2 nd Wash	1050	16.4	17220	88.31	14.21	84
3 rd Wash	1060	13.2	13992	71.75	11.54	108
Final Solid	14885.5*	5.1	75916	389.31	62.64	132
Total	-	-	121198	621.52	100	132

Final solid dry weight: 195 grams

5 Moisture Content of final solid culture: $(10-1.31)/10 \times 100\% = 86.9\%$

*Volume = $(195/13.1\%/10) \times 100 \text{ ml} = 14885.5 \text{ ml}$

10 Thus, it is seen that the foregoing general objects have been satisfied. A method for cultivating a biological agent has been provided, and also an apparatus useful in accomplishing same.

WHAT IS CLAIMED IS:

1. A culturing apparatus comprising:
a vessel having a wall defining an interior space;
5 a gas-permeable solid phase culturing bed disposed within said interior space, said bed comprising a substrate including a biological agent disposed thereon, said substrate being biologically conducive for the growth of said biological agent;
10 a gas inlet for allowing gas into said vessel; and
a gas inlet valve fluidically coupled to said gas inlet and being operable between a closed state and at least one open state thereby permitting the selective introduction of gas through said gas inlet into said
15 vessel under pressure sufficient to cause gas to flow through at least a portion of said bed.
2. The apparatus of claim 1, said substrate comprising discrete plural packings, said substrate
20 packings being packed in said bed with sufficient void volume to allow gas permeability.
3. The apparatus of claim 1, wherein said interior space includes a first void proximal a first
25 boundary of said bed and a second void proximal a second boundary of said bed, said gas inlet communicating with said vessel at said first void thereby permitting the imposition of a pressure differential between said first void and said second void whereby gas flows from said
30 first void through said first boundary, through an intermediate portion of said bed, and through said second boundary to said second void.

4. The apparatus of claim 3, said gas inlet communicating with a source of compressed gas, said vessel being selectively pressurizable via the introduction of compressed gas through said gas inlet.

5

5. The apparatus of claim 4, wherein said source of compressed gas is an air compressor.

6. The apparatus of claim 4, wherein said source
10 of compressed gas is an oxygen tank.

7. The apparatus of claim 4, further including a gas outlet fluidically communicating with said vessel at one of said first and second voids, and a gas outlet
15 valve fluidically coupled to said gas outlet, said gas outlet valve being operable between a closed state and at least one open state thereby permitting selective release of gas from said vessel through said gas outlet.

8. The apparatus of claim 7, further comprising a
20 gas outlet valve controller for generating signals for modifying the state of said gas outlet valve, and a gas outlet valve actuator responsive to said signals and modifying the state of said gas outlet valve in response
25 thereto.

9. The apparatus of claim 8, said gas outlet valve controller comprising a timer, said timer generating a signal for opening said gas outlet valve after the
30 passage of a first length of time.

10. The apparatus of claim 9, said timer subsequently generating a signal for closing said gas

outlet valve after the passage of a second length of time.

11. The apparatus of claim 8, further including a
5 temperature sensor measuring a temperature within said
bed, said gas outlet valve controller communicating with
said temperature sensor and generating a signal for
opening said gas outlet valve when said temperature has
reached a first reference temperature.

10

12. The apparatus of claim 11, said gas outlet
valve controller subsequently generating a signal for
closing said gas outlet valve when said temperature has
reached a second reference temperature.

15

13. The apparatus of claim 8, further including a
pressure sensor measuring a pressure within said vessel,
said gas outlet valve controller communicating with said
pressure sensor and generating a signal for opening said
20 gas outlet valve when said pressure has reached a first
reference pressure.

14. The apparatus of claim 13, said gas outlet
valve controller subsequently generating a signal for
25 closing said gas outlet valve when said pressure has
reached a second reference temperature.

15. The apparatus of claim 7, further comprising a
gas inlet valve controller for generating signals for
30 modifying the state of said gas inlet valve, and a gas
inlet valve actuator responsive to said signals and
modifying the state of said gas inlet valve in response
thereto.

16. The apparatus of claim 15, said gas inlet valve controller comprising a timer, said timer generating a signal for closing said gas inlet valve after the passage
5 of a first length of time.

17. The apparatus of claim 16, said timer subsequently generating a signal for opening said gas inlet valve after the passage of a second length of time.
10

18. The apparatus of claim 15, further including a temperature sensor measuring a temperature within said bed, said gas inlet valve controller communicating with said temperature sensor and generating a signal for
15 closing said gas inlet valve when said temperature has reached a first reference temperature.

19. The apparatus of claim 18, said gas inlet valve controller subsequently generating a signal for opening
20 said gas inlet valve when said temperature has reached a second reference temperature.

20. The apparatus of claim 15, further including a pressure sensor measuring a pressure within said vessel,
25 said gas inlet valve controller communicating with said pressure sensor and generating a signal for closing said gas inlet valve when a pressure within said vessel has reached a first reference pressure.

30 21. The apparatus of claim 20, said gas inlet valve controller subsequently generating a signal for opening said gas inlet valve when said pressure has reached a second reference pressure.

22. The apparatus of claim 3, further including a second gas inlet, said second gas inlet fluidically communicating with said vessel at said second void.

5

23. The apparatus of claim 22, said vessel further including a second gas inlet valve and a second gas inlet valve controller for generating signals for modifying the state of said second gas inlet valve, and a second gas inlet valve actuator responsive to said signals and modifying the state of said second gas inlet valve in response thereto.

24. The apparatus of claim 1, wherein said interior space includes a head space proximal a first boundary of said bed, said vessel including a gas inlet fluidically communicating with said vessel at said head space.

25. The apparatus of claim 3, wherein said biological agent is selected from the group consisting of bacteria, yeasts, molds, fungi, and combinations thereof.

26. A culturing apparatus comprising:
a vessel having a wall defining an interior space;
25 a gas-permeable solid phase culturing bed disposed within said interior space, said bed comprising a substrate including a biological agent disposed thereon, said substrate being biologically conducive for the growth of said biological agent;
30 a gas inlet permitting the introduction of gas into said vessel;
a gas outlet; and

a gas outlet valve fluidically coupled to said gas outlet and being operable between a closed state and at least one open state thereby permitting the selective exhaustion of gas from said vessel through said gas
5 outlet.

27. The apparatus of claim 26, said substrate comprising discrete plural packings, said substrate packings being packed in said bed with sufficient void
10 volume to allow gas permeability.

28. The apparatus of claim 26, wherein said interior space includes a first void proximal a first boundary of said bed and a second void proximal a second
15 boundary of said bed, said gas inlet communicating with said vessel at said first void thereby permitting the imposition of a pressure differential between said first void and said second void whereby gas flows from said first void through said first boundary, through an
20 intermediate portion of said bed, and through said second boundary to said second void.

29. The apparatus of claim 28, said gas inlet communicating with a source of compressed gas, said
25 vessel being selectively pressurizable via the introduction of compressed gas through said gas inlet.

30. The apparatus of claim 29, wherein said source of compressed gas is an air compressor.

30

31. The apparatus of claim 29, wherein said source of compressed gas is an oxygen tank.

32. The apparatus of claim 29, said gas outlet communicating with said vessel at one of said first and second voids.

5 33. The apparatus of claim 26, further comprising a gas outlet valve controller for generating signals for modifying the state of said gas outlet valve, and a gas outlet valve actuator responsive to said signals and modifying the state of said gas outlet valve in response
10 thereto.

34. The apparatus of claim 33, said gas outlet valve controller comprising a timer, said timer generating a signal for opening said gas outlet valve
15 after the passage of a first length of time.

35. The apparatus of claim 34, said timer subsequently generating a signal for closing said gas outlet valve after the passage of a second length of
20 time.

36. The apparatus of claim 33, further including a temperature sensor measuring a temperature within said bed, said gas outlet valve controller communicating with
25 said temperature sensor and generating a signal for opening said gas outlet valve when said temperature has reached a first reference temperature.

37. The apparatus of claim 36, said gas outlet
30 valve controller subsequently generating a signal for closing said gas outlet valve when said temperature has reached a second reference temperature.

38. The apparatus of claim 33, further including a pressure sensor measuring a pressure within said vessel, said gas outlet valve controller communicating with said pressure sensor and generating a signal for opening said gas outlet valve when said pressure has reached a first reference pressure.

39. The apparatus of claim 38, said gas outlet valve controller subsequently generating a signal for closing said gas outlet valve when said pressure has reached a second reference temperature.

40. The apparatus of claim 26, further comprising a gas inlet valve fluidically coupled to said gas inlet valve and communicating with said vessel at one of said first and second voids, said gas inlet valve being operable between a closed state and at least one open state and further comprising a gas inlet valve controller for generating signals for modifying the state of said gas inlet valve, and a gas inlet valve actuator responsive to said signals and modifying the state of said gas inlet valve in response thereto, the apparatus further comprising a gas outlet valve controller for generating signals for modifying the state of said gas outlet valve, and a gas outlet valve actuator responsive to said signals and modifying the state of said gas outlet valve in response thereto.

41. A method of cultivating a biological agent comprising the steps of:

providing a gas-permeable solid phase culturing bed, said bed comprising a substrate including a biological

agent disposed thereon, said substrate being biologically conducive for the growth of said biological agent;

flowing a gas through said bed thereby causing said gas to come into contact with at least a portion of said
5 biological agent; and

subsequently recovering at least a portion of said biological agent.

42. The method of claim 41, wherein said gas is an
10 oxygen-containing gas.

43. The method of claim 41, wherein said biological agent is selected from the group consisting of bacteria, yeasts, molds, fungi, and combinations thereof.

15

44. The method of claim 41, wherein said biological agent is selected from the group consisting of cellulase enzyme producing biological agents.

20 45. The method of claim 44, wherein said substrate is selected from the group consisting of whole grain rice, wheat bran, and cracked corn.

46. The method of claim 44, wherein said biological
25 agent is selected from the group consisting of *Trichoderma* and *A. niger*.

47. The method of claim 41, said bed being disposed within an interior space defined by a wall of a vessel,
30 said vessel including a gas inlet and a gas inlet valve fluidically coupled thereto, said gas inlet valve being operable between a closed state and at least one open state thereby permitting the selective introduction of

gas through said gas inlet into said vessel under pressure sufficient to cause gas to flow through at least a portion of said bed, said method including the step of opening said gas inlet valve.

5

48. The method of claim 47, wherein said interior space includes a first void proximal a first boundary of said bed and a second void proximal a second boundary of said bed, said gas inlet communicating with said vessel at said first void thereby permitting the imposition of a pressure differential between said first void and said second void, said method including the step of introducing gas into said vessel through said gas inlet whereby gas flows from said first void through said first boundary, through an intermediate portion of said bed, and through said second boundary to said second void.

49. The method of claim 48, said vessel further including a gas outlet and a gas outlet valve fluidically coupled thereto, the method comprising the steps of:
determining a reference temperature;
measuring a reference temperature within said bed;
and
opening said gas outlet valve if said temperature has reached or exceeded said reference temperature.

50. The method of claim 49, further comprising the steps of:
determining a second reference temperature;
after opening said gas outlet valve, measuring a reference temperature within said bed; and
closing said gas outlet valve if said temperature has fallen to or below said second reference temperature.

51. The method of claim 48, said vessel further including a gas outlet and a gas outlet valve fluidically coupled thereto, the method comprising the steps of:

5 determining a reference pressure;
 measuring a reference pressure within said bed;
 and

 opening said gas outlet valve if said pressure has reached or exceeded said reference pressure.

10

52. The method of claim 51, further comprising the steps of:

 determining a second reference pressure;
 after opening said gas outlet valves, measuring a
15 pressure within said bed; and

 closing said gas outlet valve if said pressure has fallen to or below said second reference pressure.

53. The method of claim 48, said vessel further
20 includes a gas outlet and a gas outlet valve fluidically coupled thereto, and a gas outlet valve controller for generating signals for modifying the state of said gas outlet valve, and a gas outlet valve actuator responsive to said signals and modifying the state of said gas
25 outlet valve in response thereto, said method including the step of generating a signal for opening said gas outlet valve.

54. The method of claim 53, wherein said gas outlet
30 valve controller comprises a timer, said method including the steps of:

 introducing gas through said gas inlet to thereby pressurize said vessel;

after the passage of a first length of time,
generating a signal for opening said gas outlet valve.

55. The method of claim 54, further comprising the
5 step of generating a signal for closing said gas outlet
valve after the passage of a second length of time.

56. The method of claim 53, wherein said vessel
includes a pressure sensor measuring a pressure
10 therewithin, said pressure sensor communicating with said
controller, said method including the steps of:

introducing gas through said gas inlet to thereby
pressurize said vessel;

after a pressure within said vessel has reached a
15 first reference pressure, generating a signal for opening
said gas outlet valve.

57. The method of claim 56, further comprising the
step of generating a signal for closing said gas outlet
20 valve after said pressure has decreased to a second
reference pressure.

58. The method of claim 48, wherein said vessel
further includes a second gas inlet communicating with
25 said vessel at said second void, said vessel further
including a second gas inlet valve, the method comprising
the steps of:

introducing gas into said vessel through said gas
inlet valve and through said second gas inlet valve;
30 determining a reference temperature;
measuring a temperature within said bed; and

closing said second gas inlet valve if said temperature has reached or exceeded said reference temperature.

5 59. The method of claim 58, further comprising the steps of:

 determining a second reference temperature;

 after closing said second gas inlet valve, measuring a temperature within said bed; and

10 operating said gas inlet valve if said temperature has fallen to or below said second reference temperature.

 60. The method of claim 48, wherein said vessel further includes a second gas inlet communicating with
15 said vessel at said second void, said vessel further including a second gas inlet valve and a gas inlet valve controller for generating signals for modifying the state of said second gas inlet valve, and a second gas inlet
20 valve actuator responsive to said signals and modifying the state of said second gas inlet valve in response thereto, said method including the steps of:

 introducing gas into said vessel via said first gas inlet and said second gas inlet; and

25 subsequently generating a signal for closing said second gas inlet valve.

 61. The method of claim 41, wherein such step of recovering said biological agent comprises recovering a biological product produced by said biological agent.

30

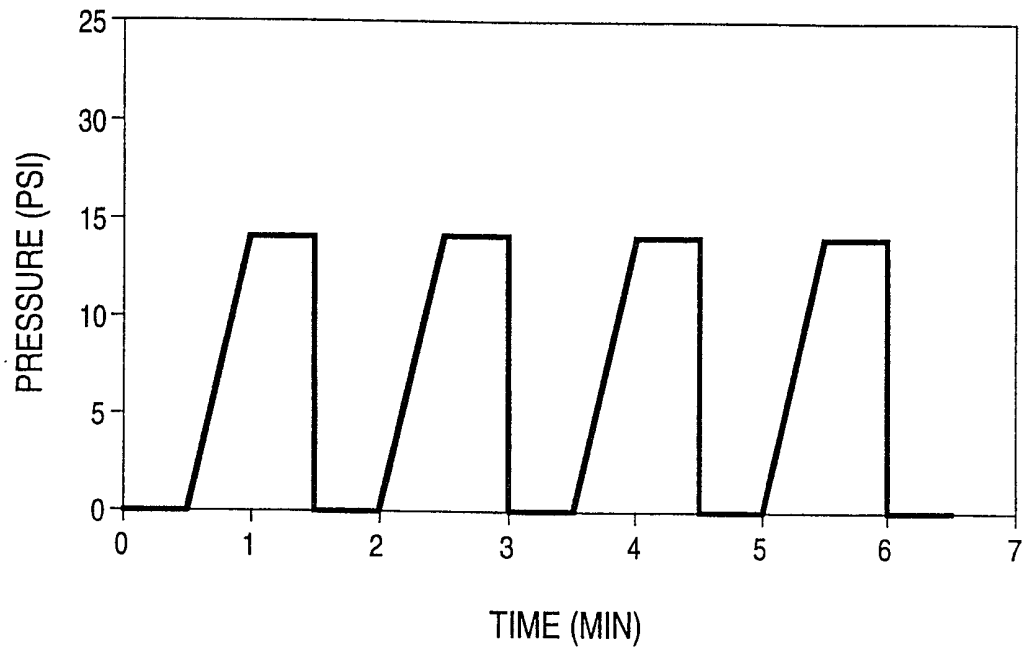
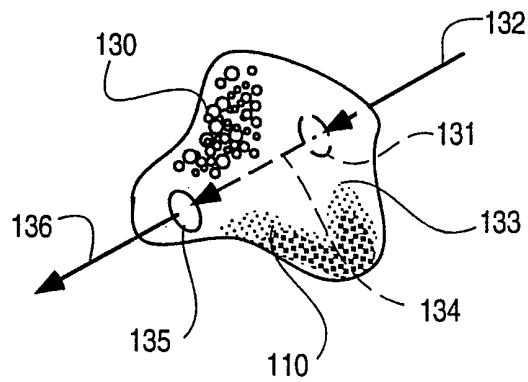
FIG. 2**FIG. 3**

Fig. 4

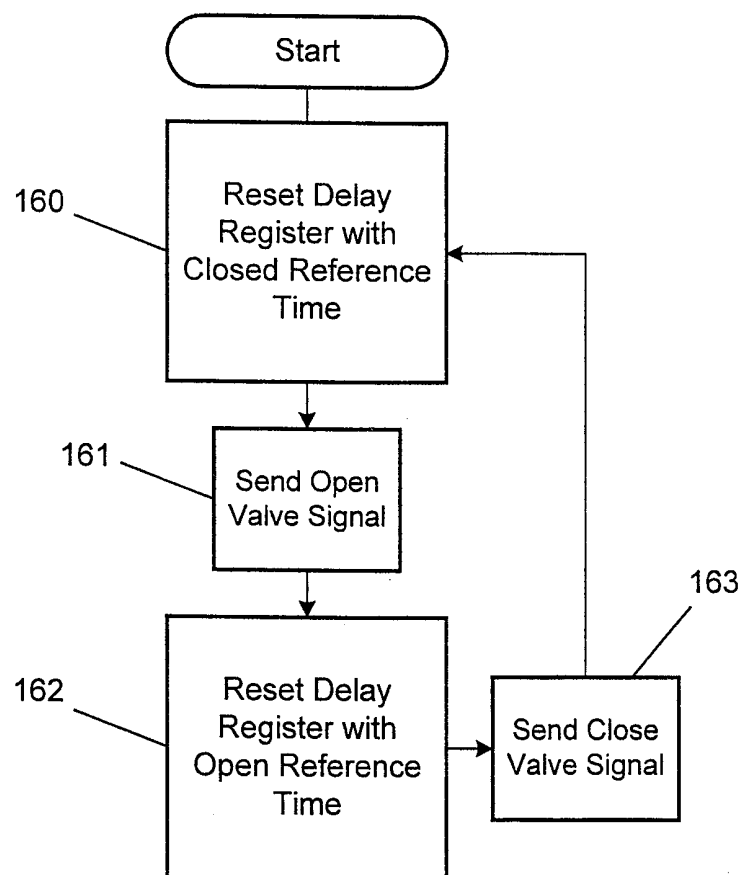


Fig. 5

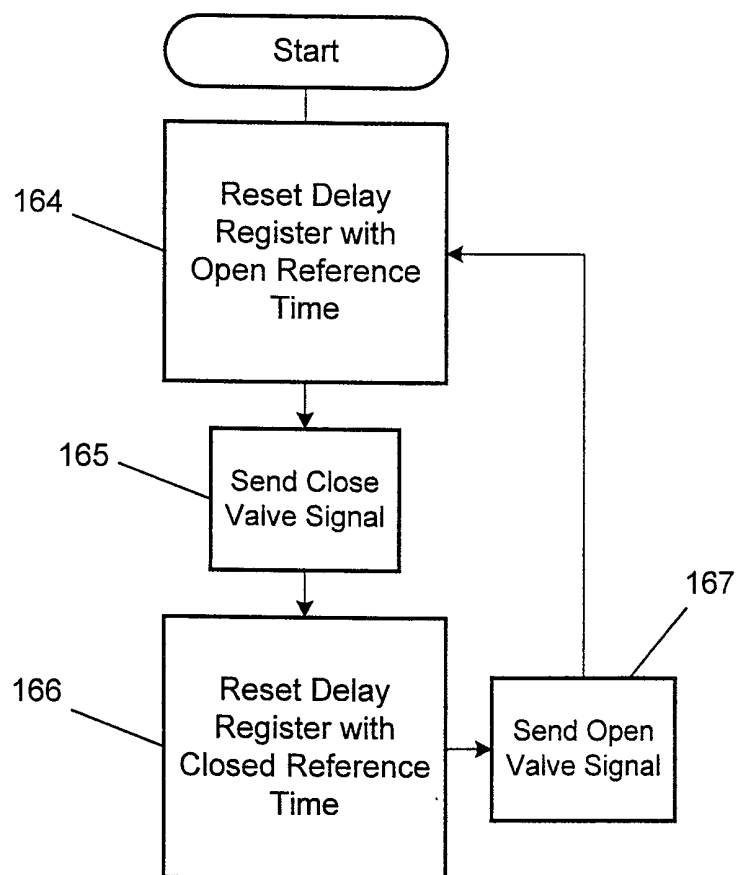


Fig. 6

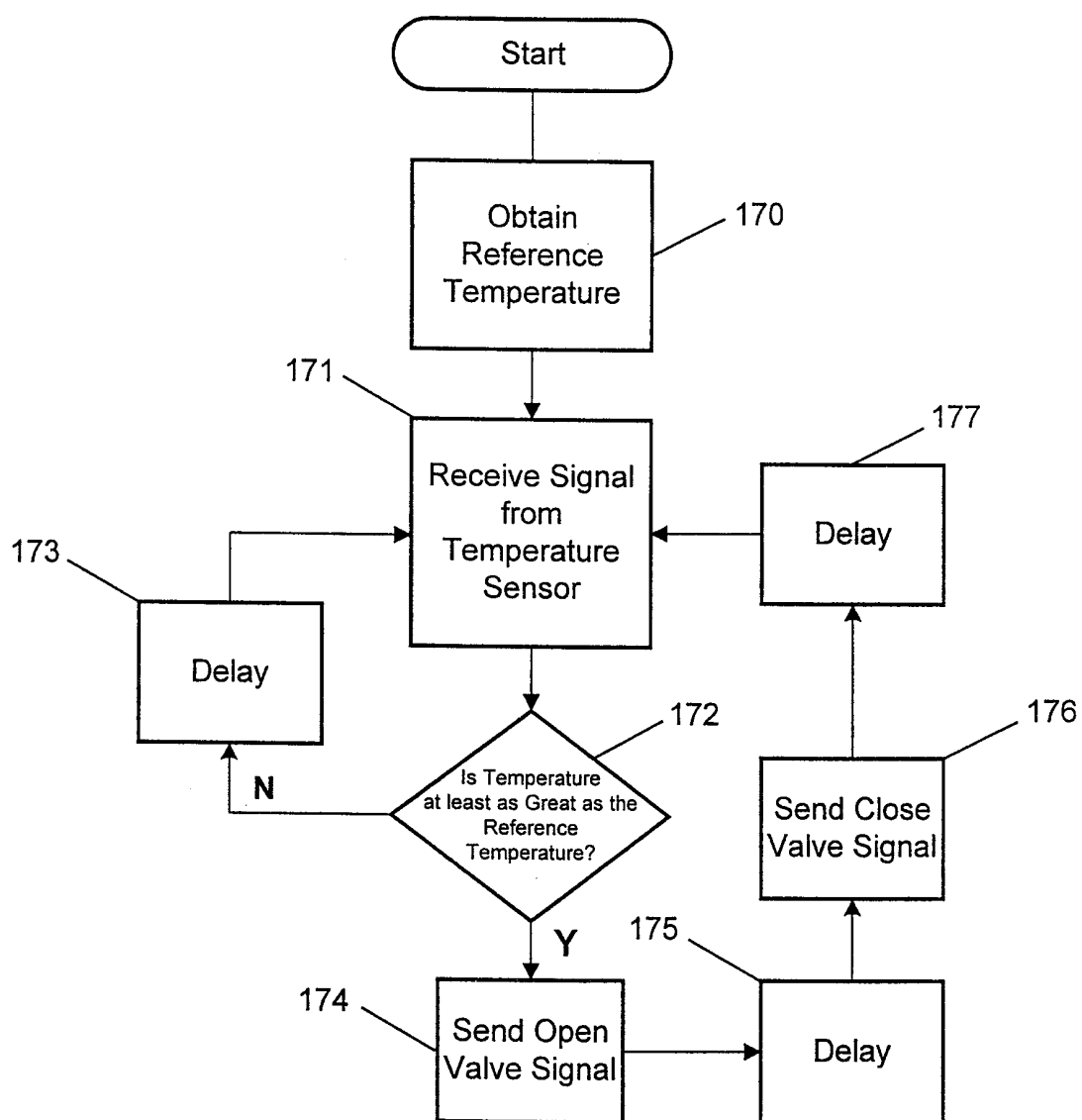


Fig. 7

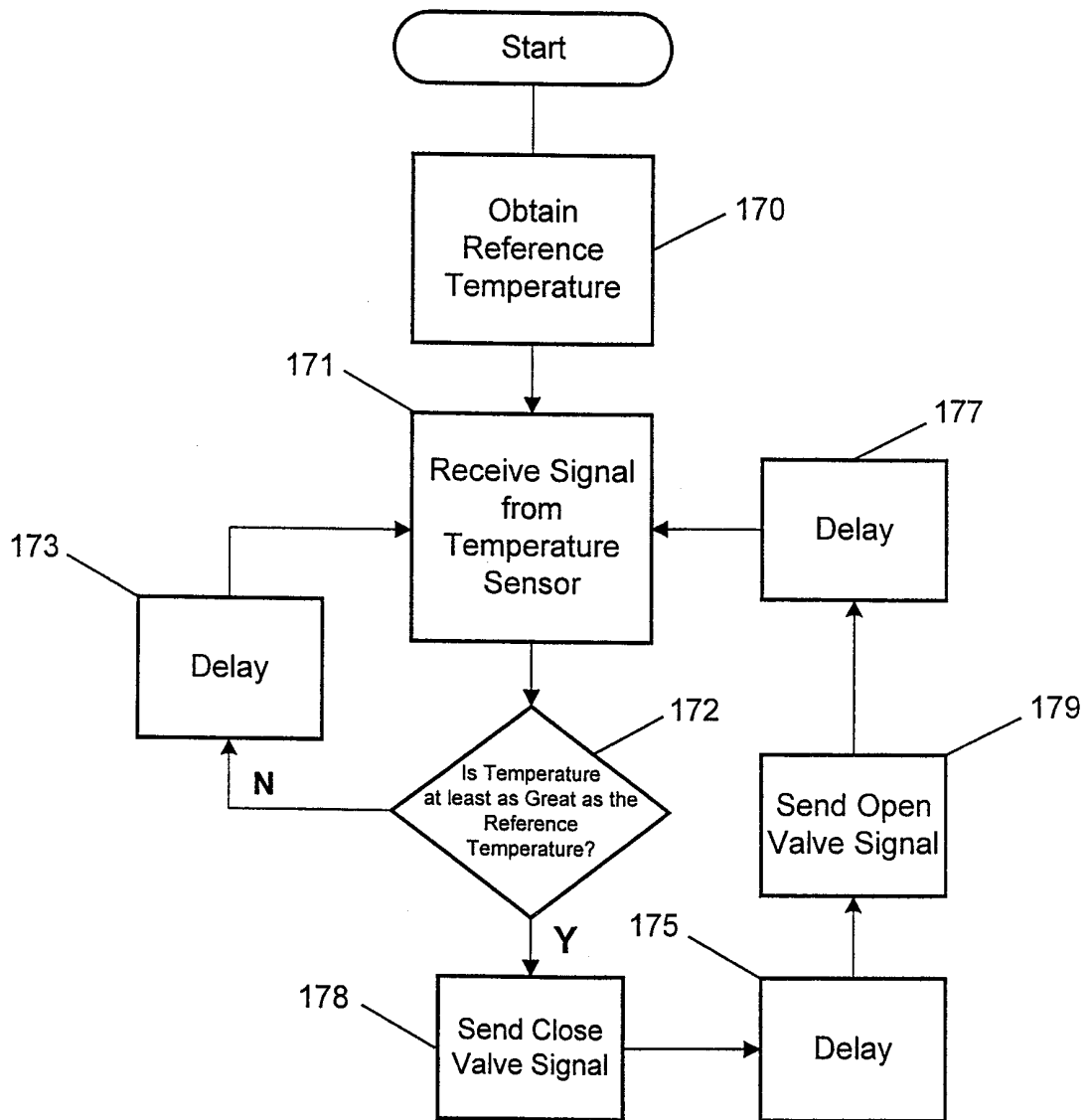


Fig. 8

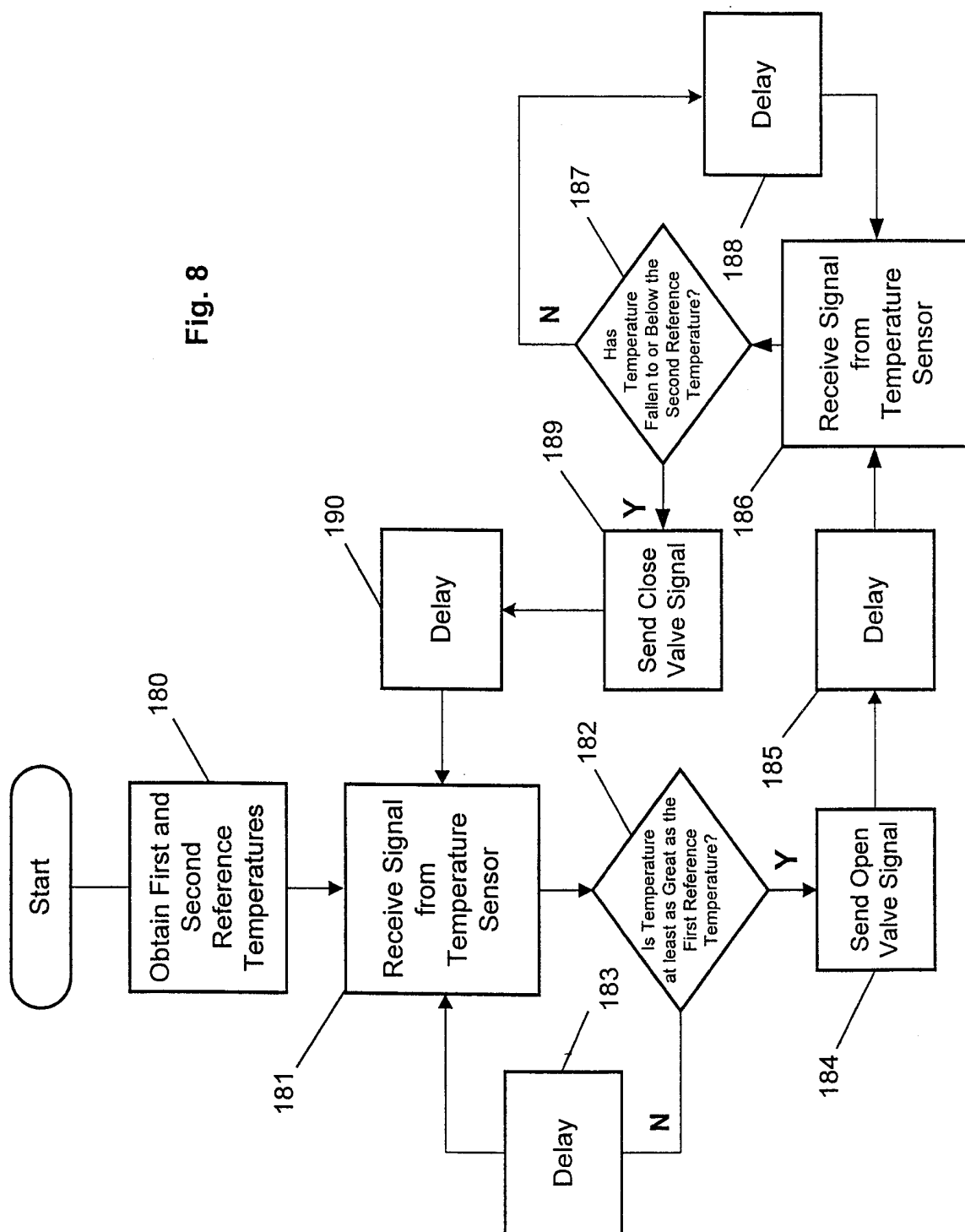


Fig. 9

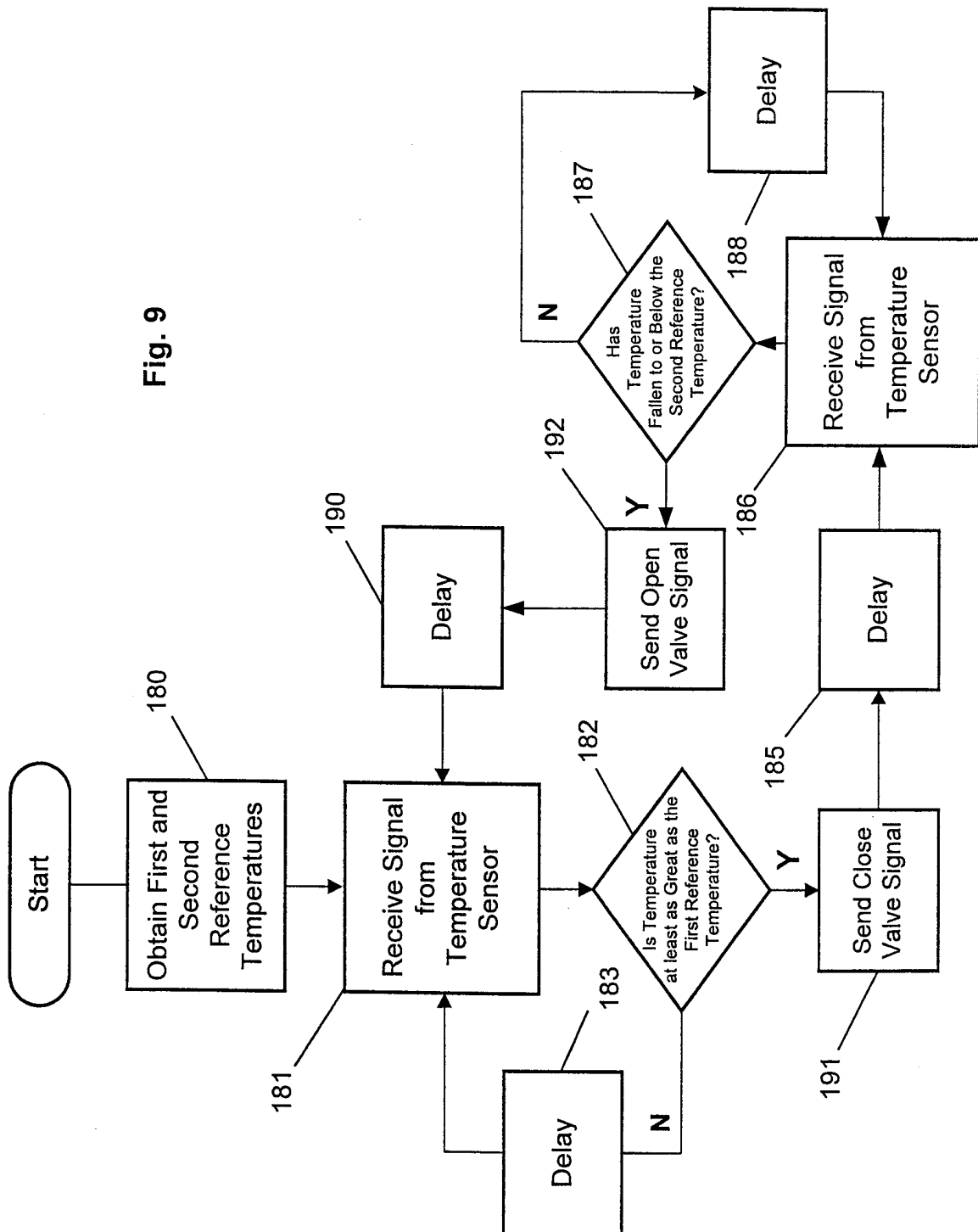


Fig. 10

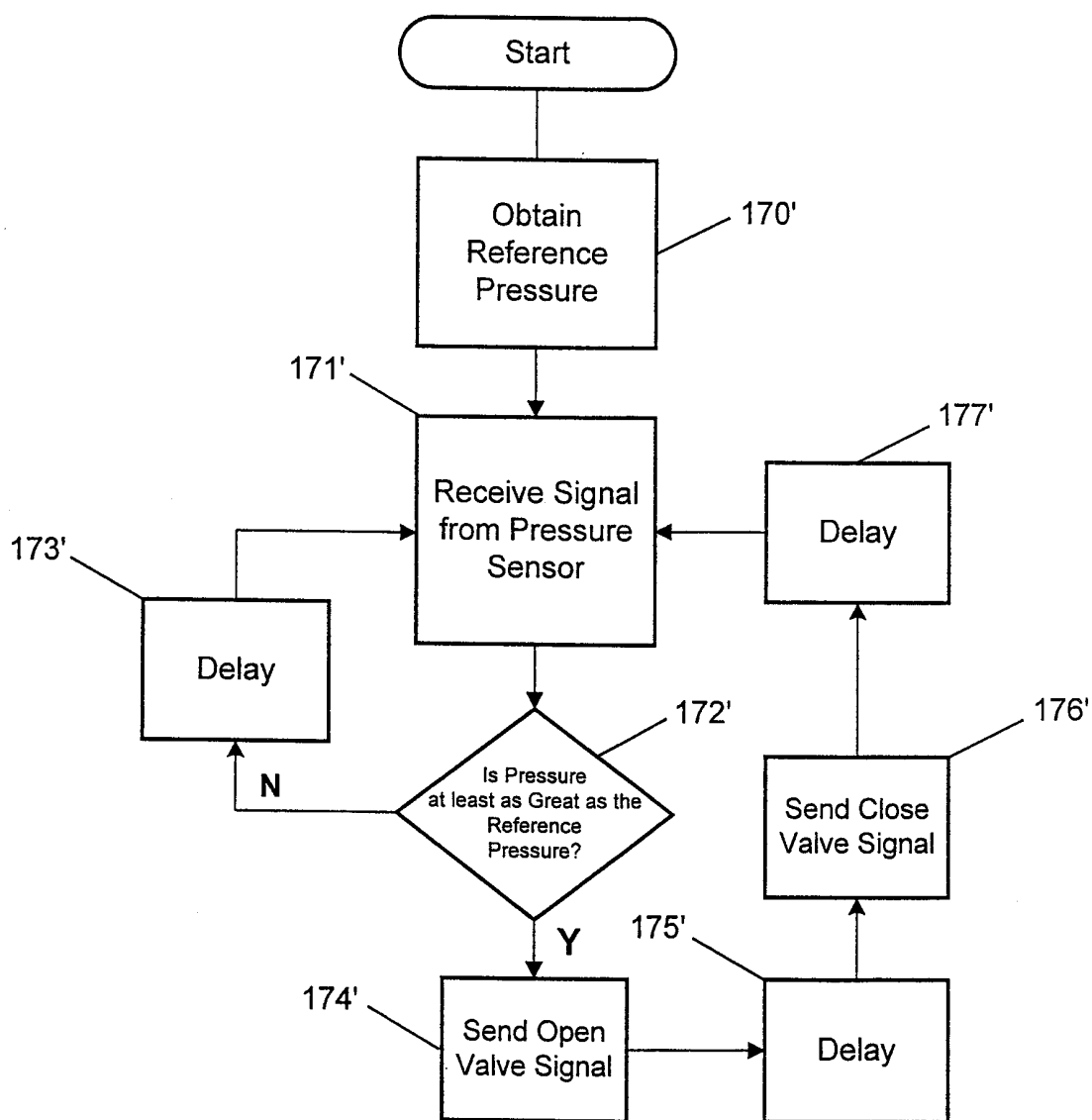


Fig. 11

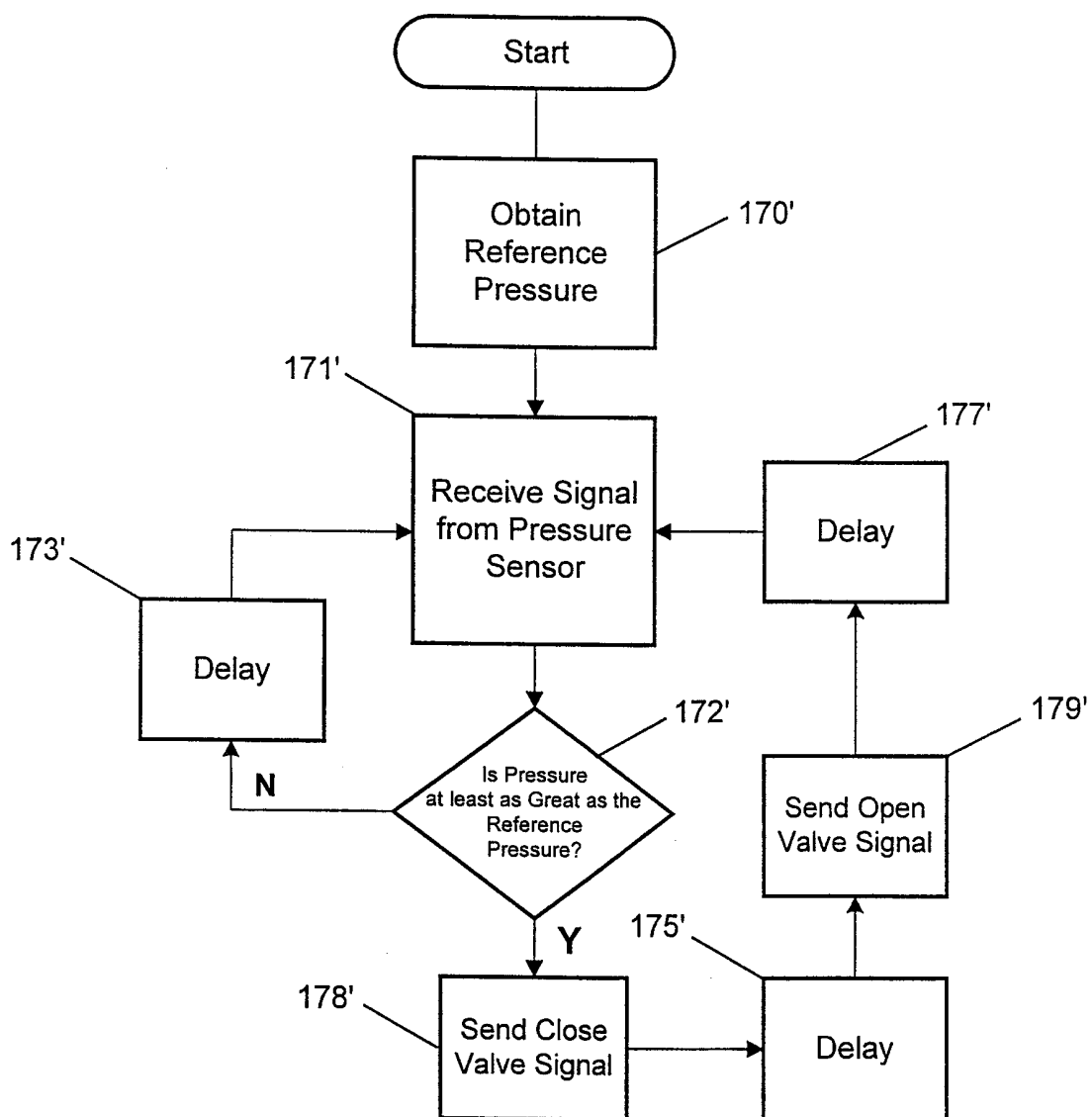


Fig. 12

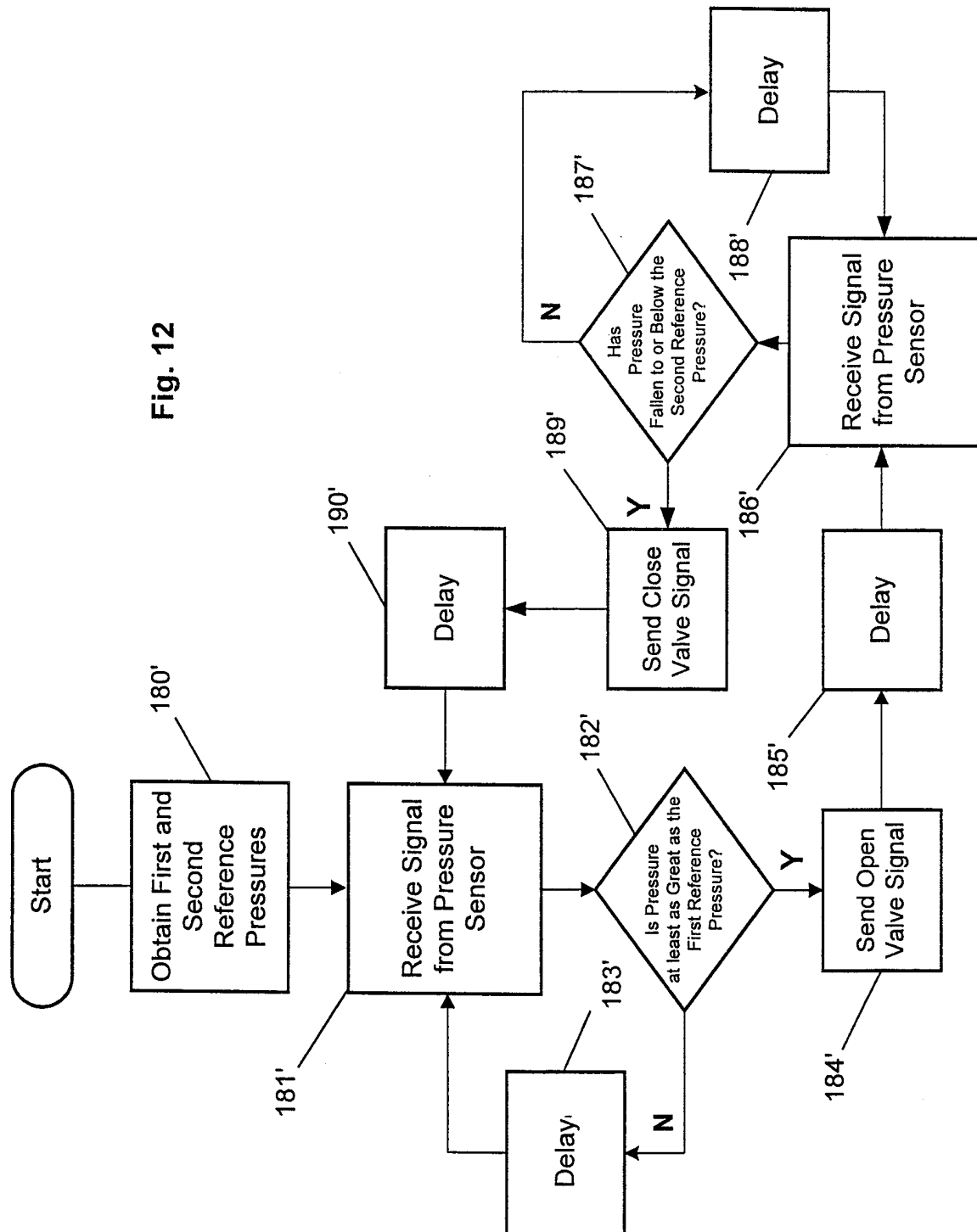
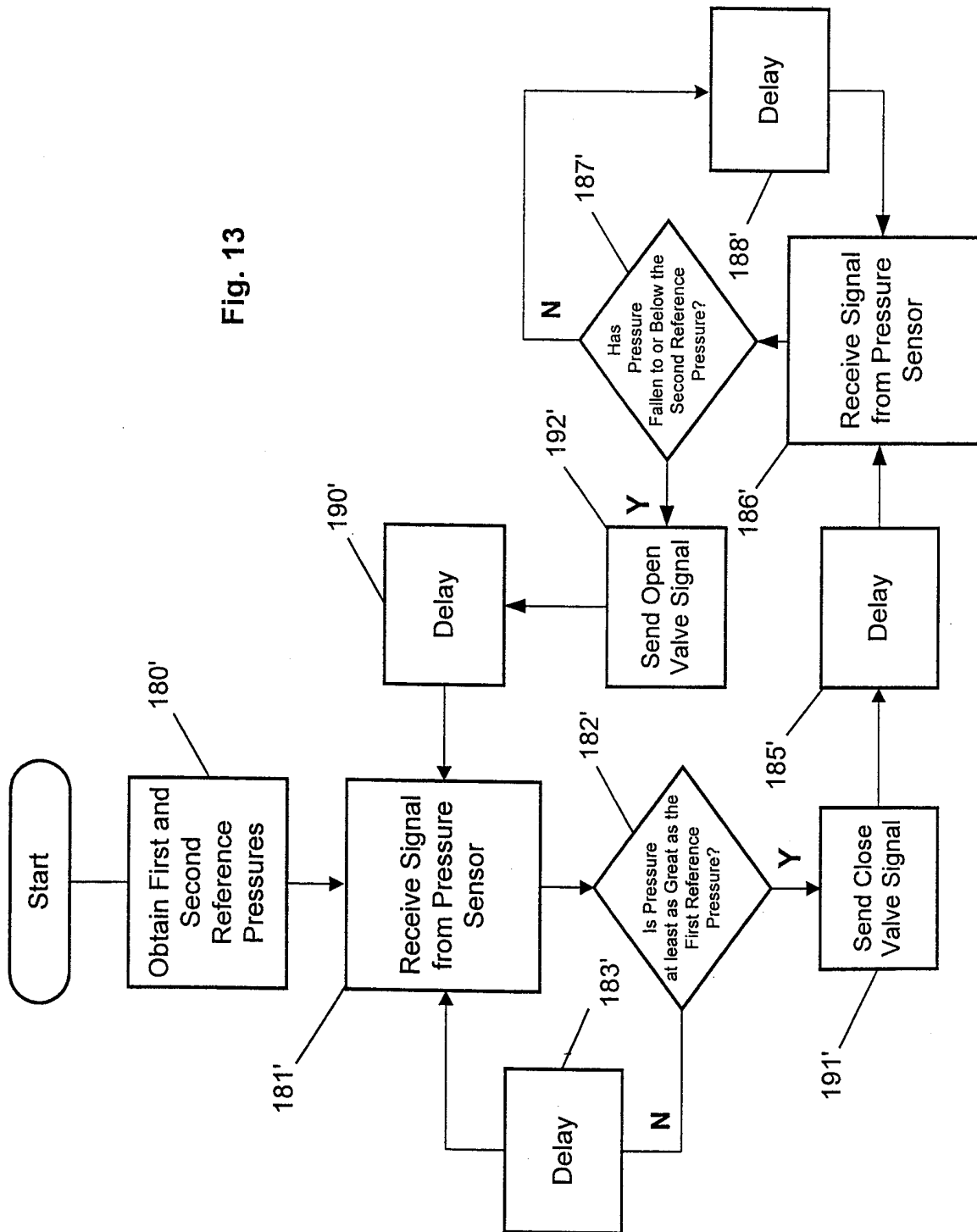


Fig. 13



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US99/08552

A. CLASSIFICATION OF SUBJECT MATTER

C 12 M 1/04, C 12 M 1/38

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)

C 12 M

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

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CH 647546 A5 (SOCIETE SAPS ANTICORROSION) 31 January 1985, abstract. --	1, 26
A	US 3846245 A (KONDIS et al.) 05 November 1974, fig., column 7, line 63 - column 8, line 75. --	1, 26, 41
A	DE 2741940 A1 (LINDE AG) 29 March 1979, fig., page 9, line 21 - page 12, line 7. ----	1, 8- 10, 26, 33-35

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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Date of the actual completion of the international search

19 August 1999

Date of mailing of the international search report

16.09.99

Name and mailing address of the ISA

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Fax (+31-70) 340-3016

Authorized officer

WOLF e.h.

ANHANG

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

ANNEX

to the International Search
Report to the International Patent
Application No.

ANNEXE

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/US 99/08552 SAE 232343

In diesem Anhang sind die Mitglieder
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Im Recherchenbericht angeführtes Patentdokument in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
CH A	647546	31-01-1985	BE A1 889185 11-12-1981 CA A1 1170378 03-07-1984 DE A1 3122389 27-05-1982 DE C2 3122389 14-12-1989 FR A1 2484447 18-12-1981 FR B1 2484447 17-02-1984 GB A1 2077712 23-12-1981 GB B2 2077712 08-06-1983
US A	3846245	05-11-1974	keine - none - rien
DE A1	2741940	29-03-1979	AT A 8995/77 15-02-1980 AT B 358514 10-09-1980 AU A1 39914/78 20-03-1980 AU B2 519651 17-12-1981 BR A 7806067 02-05-1979 DE C2 2741940 26-06-1986 FR A1 2403383 13-04-1979 FR B1 2403383 21-10-1983 ZA A 7805264 29-08-1979