A control system for an actively cooled cryogenic biological preservation unit wherein vapor flow into or out of the unit or a proxy thereof is sensed by a flow sensor signaling a controller which modulates cryocooler refrigeration into the unit.
CONTROL SYSTEM FOR ACTIVELY COOLED CRYOGENIC BIOLOGICAL PRESERVATION UNIT

TECHNICAL FIELD

[0001] This invention relates generally to preservation of biological samples and, more particularly, to preservation of biological samples at cryogenic temperatures.

BACKGROUND ART

[0002] There is a growing trend toward cryogenic storage of biological samples at temperatures below 140 K. This trend is driven by the fact that little to no sample degradation occurs below the sample glass transition temperature which is about 140 K. Conventional cryogenic biological sample preservation units store biological samples at temperatures below 140 K use liquid nitrogen to keep the biological samples cold. These units typically store the samples within a vacuum insulated space above a pool of liquid nitrogen or immersed within the pool of liquid nitrogen. The liquid nitrogen needs to be periodically replenished due to loss of nitrogen vapor from the unit. This is costly, not only because of the cost of the nitrogen, but also because of the complicated procedures required to handle the liquid nitrogen.

[0003] An improvement to conventional cryogenic biological preservation units involves active cooling wherein refrigeration is provided to the unit to maintain the cryogenic conditions established by the liquid nitrogen. It is desirable to minimize loss of liquid nitrogen from an actively cooled preservation unit to reduce ambient contamination migration into an actively cooled preservation unit.

SUMMARY OF THE INVENTION

[0004] One aspect of the invention is:

[0005] An actively cooled cryogenic biological preservation unit comprising:

[0006] (A) an insulated vessel having a vessel interior, and a cryocooler positioned to provide refrigeration to the insulated container;

[0007] (B) an exhaust line in communication with the vessel interior, and a flow sensor on the exhaust line; and

[0008] (C) a controller, means for passing a signal from the flow sensor to the controller, and means for passing a signal from the controller to the cryocooler.

[0009] Another aspect of the present invention is:

[0010] A method for operating an actively cooled cryogenic biological preservation unit comprising:

[0011] (A) providing refrigeration to the interior of an insulated vessel having an exhaust line in communication with the vessel interior;

[0012] (B) sensing the flow of gas within the exhaust line; and

[0013] (C) adjusting the amount of refrigeration provided to the vessel interior based on the gas flow sensing.

[0014] As used herein the term “cryocooler” means a refrigeration which can produce refrigeration below 193 K for the purpose of cooling biological samples.

[0015] As used herein the term “cold head” means the portion of the cryocooler containing the cold heat exchanger, aftercooler and regenerator.

[0016] As used herein the term “cold finger” means a portion of a cold head that is configured such that the cold heat exchanger is located at one end of the cold head. The cold finger refers to the portion of the cold head with this configuration that, in operation, is at a temperature below that of the aftercooler.

[0017] As used herein the term “biological sample” means an organic material. Some examples of biological samples are proteins, blood platelets, cartilage and heart valves.

[0018] As used herein the term “controller” means a physical device that monitors a measured quantity of a system (the controlled variable), compares it with a desired value (the setpoint), and actuates the system in such a way as to either cause the controlled variable to eventually equal the desired setpoint or prevent the controlled variable from deviating from the setpoint by more than a predetermined amount.

[0019] As used herein the term “proportional integral derivative controller” means a controller that utilizes an algorithm based on an error signal (the difference between the controlled variable and the desired setpoint), the integral of the error signal, and the rate of change of the error signal, to determine how to actuate the system to either cause the controlled variable to eventually equal the desired setpoint or prevent the controlled variable from deviating from the setpoint by more than a predetermined amount.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. 1 is a cross sectional representation of one preferred embodiment of the actively cooled cryogenic biological preservation unit of this invention.

[0021] FIG. 2 is a schematic representation of one preferred control system which may be used in the practice of the actively cooled cryogenic biological preservation unit of this invention.

[0022] FIG. 3 is a schematic representation of another preferred control system which may be used in the practice of this invention.

[0023] FIG. 4 is a schematic representation of yet another preferred control system which may be used in the practice of this invention.

DETAILED DESCRIPTION

[0024] In the practice of this invention a flow sensor is used to provide feedback for the active cooling system control. When gas flow is out of the cryogenic biological preservation unit, the control system serves to increase the amount of refrigeration provided into the unit, thus reducing the amount of cryogen, e.g., liquid nitrogen, flowing out of the unit as vapor. When gas flow into the unit, the control system serves to decrease the amount of refrigeration provided into the unit, either by reducing the cryocooler activity and/or increasing heat input to the unit, thus reducing or eliminating the flow of ambient air and contaminants into the unit.

[0025] The invention will be described in greater detail with reference to the Drawings. Referring now to FIG. 1,
there is shown a cryogenic biological preservation unit comprising an insulated vessel having a vessel wall 9 and having insulation, typically vacuum insulation, 12 adjacent the inside of vessel wall 9. Vessel wall 1 and insulation 12 define the vessel interior or storage space 10. In the lower portion of vessel interior 10 is a pool of liquid nitrogen 8.

[0026] Within vessel interior 10 and preferably above liquid nitrogen pool 8 there is stored at least one biological sample. In FIG. 1 there is illustrated in representational form a plurality of biological samples within vapor space 11. In general the cryogenic biological preservation unit of this invention will have a diameter within the range of from 30 to 60 inches and a height within the range of from 45 to 75 inches. Depending upon the size of the biological samples and upon the type of rack system used, the cryogenic biological preservation unit of this invention can accommodate or store up to 15,000 to 80,000 biological samples in 1-2 ml plastic vials. Large items such as blood bags and organs can also be stored.

[0027] The cryogenic biological preservation unit of this invention has an opening 13 which allows access to the vessel interior 10 from outside the vessel and through which biological samples are put into and removed from the vessel interior. Within opening 13 there is positioned insulated lid 7 which is typically insulated using a closed cell foam such as expanded poly styrene, and which is positioned in opening 13 when access to vessel interior 10 is not desired.

[0028] Any suitable cryocooler may be used in the practice of this invention. Among such cryocoolers one can name Stirling cryocoolers, Gilford-McMahon cryocoolers and pulse tube refrigerators. A pulse tube refrigerator is a closed refrigeration system that oscillates a working gas in a closed cycle and in so doing transfers a heat load from a cold section to a hot section. The frequency and phasing of the oscillations is determined by the configuration of the system. The driver or pressure wave generator may be a piston or some other mechanical compression device, or an acoustic or thermoacoustic wave generation device, or any other suitable device for providing a pulse or compression wave to a working gas. That is, the pressure wave generator delivers energy to the working gas within the pulse tube causing pressure and velocity oscillations. Helium is the preferred working gas; however any effective working gas may be used in the pulse tube refrigerator and among such one can name nitrogen, oxygen, argon and neon or mixtures containing one or more thereof such as air.

[0029] The oscillating working gas is preferably cooled in an aftercooler and then in a regenerator as it moves toward the cold end. The geometry and pulsing configuration of the pulse tube refrigeration system is such that the oscillating working gas in the cold head expands for some fraction of the pulsing cycle and heat is absorbed by the working gas by indirect heat exchange which provides refrigeration to the vessel interior. Preferably the pulse tube refrigeration system employs an inerter tube and reservoir to maintain the gas displacement and pressure pulses in appropriate phases. The size of the reservoir is sufficiently large so that essentially very little pressure oscillation occurs in it during the oscillating flow.

[0030] The cryocooler 1 components include the mechanical compression equipment (pressure wave generator), the inerter tube and reservoir, the final heat rejection system and the electrical components required to drive and control the cryocooler. Electrical energy is primarily converted into acoustic energy in the pressure wave generator. This acoustic energy is transfered by the oscillating working gas to the cold head via a transfer tube. The transfer tube connects the pressure wave generator to the aftercooler located at the warm end of the cold head, where heat is removed as previously described. The cryocooler is controlled to provide varying amounts of refrigeration to the cold end of the cold finger 5 depending on the conditions in the cryogenic biological preservation unit vessel interior 10. This is accomplished by modulating the acoustic power output from the pressure wave generator by varying the voltage and thus the electrical power supplied.

[0031] In the embodiment of the invention illustrated in FIG. 1, cold finger 5 penetrates into vessel interior 10 and provides refrigeration directly to the vessel interior. The refrigeration cools and condenses nitrogen vapor within the upper portion of the vessel interior 10 as will be more fully described below, thus eliminating the need to replenish the liquid nitrogen from outside the unit and thereby minimizing costly and complicated liquid nitrogen handling procedures and systems. The condensed nitrogen falls by gravity to the liquid nitrogen pool 8 in the lower portion of the vessel interior.

[0032] The temperature at the lowest level of the sample storage within the vessel interior may be as low as 77 K and is generally within the range of from 80 to 95 K. However, the normal temperature at the upper levels of the sample storage may be within the range of from 95 to 140 K without the use of the active cooling of this invention. Samples in the top racks of conventional cryogenic biological preservation units could exceed the glass transition temperature of the biological samples when the lid is removed for access to the interior. For this reason, storage of biological samples in the upper portion of conventional cryogenic biological preservation units is often avoided. However, with the cryogenic biological preservation unit of this invention which provides cryocooler refrigeration to the upper portion of the vessel interior, biological samples may be stored in the upper portion of the vessel interior without fear of degradation due to elevated temperature. This increases the effective capacity of the unit which is another advantage of the cryogenic biological preservation unit of this invention over conventional systems. In the practice of this invention, the cryocooler will continuously recondense all or most of the nitrogen vaporized due to heat leak caused by opening and closing the vessel and by the integration of the cryocooler with the vessel itself.

[0033] There is a need to reduce the liquid nitrogen consumption for a cryogenic biological preservation unit (CBPU) while decreasing the cost to operate the unit. The use of an active cooling system vessel could still allow cryogen vapor to escape out of the vessel or allow air to infiltrate into the vessel. Air infiltration into the CBPU could cause both operational and safety problems. If air infiltrates into the CBPU, most of the components of air would be liquefied or frozen. Air liquefaction in the CBPU could cause oxygen concentration in the cryogen pool in the CBPU which could be hazardous. This is a result of air being drawn into the CBPU where most of its components would be liquefied or frozen, including oxygen. Liquid air could accumulate in the cryogen pool. During periods of cryogen
boil-off, nitrogen would be preferentially boiled from the pool leading to the concentration of oxygen in the pool. A control and instrumentation system is therefore desirable for such a system to minimize both nitrogen losses from the CBPU and contaminant infiltration into the CBPU.

[0034] The direction of flow in or out of the vessel is dependent on the minute differential pressure that exists between the vapor space of the CBPU and the atmosphere. The magnitude and sign of the differential pressure is dependent on the system heat balance and the amount of cooling delivered by the active cooling system as well as the physical resistance of the system to these flows. The normal (non-actively cooled) differential pressure for conventional CBPU’s is on the order of 0.001 inch water. The exhaust line flow caused by this differential pressure is on the order of several hundred to a few thousand cc/min. If the cooling delivered is higher than the heat leak, then the vapor space pressure will drop as the cryogen is condensed and air would be drawn in. If the cooling delivered is lower than the heat leak, then the vapor space pressure will rise, thus expelling some of the vaporized cryogen. The net cooling can be controlled to minimize the flow into and out of the vessel; however this flow is also affected by local changes in barometric pressure and drafts around the CBPU.

[0035] The flow into or out of the CBPU vessel is dependent on the minute differential pressure that exists between the vapor space of the vessel and the atmosphere. This flow normally occurs past the walls of the foam insulated lid provided in these vessels to access samples. The lids provided with these vessels therefore close and insulate the vessel, but do not seal the vessel from the atmosphere. The present invention involves a method to adjust the net cooling delivered by the cryocooler in response to a flow rate out of a small slip stream exhaust line penetrating the CBPU vessel or lid. A flow sensor 2 is provided on exhaust line 3 to measure the magnitude and direction of flow. The flow through exhaust line 3 is typically small, and cold vapor passing into exhaust line 3 warms to a temperature high enough so as not to harm or negatively affect the flow sensor 2 as the vapors pass through the sensor.

[0036] The signal from sensor 2 is sent to a proportional integral derivative (PID) controller 4. The PID controller will have a pre-programmed setpoint for this flow rate that is normally greater than zero (net outflow). This controller then continuously determines the net cooling required to achieve the exhaust line flow setpoint. It should be noted that the exhaust line sensor need not measure all of the flow out of the CBPU, but the portion of the flow measured, typically 10% and between 5 and 20% by volume is adequate to enable the function of the control system disclosed. The overall mass flow (loss) rate of the CBPU vessel can be measured and compared to the readings from the flow sensor obtained for similar conditions. The portion of flow passing through the sensor can then be determined. This portion can be assumed to be a constant over the normal range of operation.

[0037] The output PID controller is preferably used to adjust the input power to the motor driving the cryocooler 1, thereby adjusting the net cooling delivered. This adjustment can be achieved by on/off or continuously variable control means. The output of the PID controller 4 can alternately be used to activate a heater 6 attached to the cold finger 5 of the cryocooler, thereby also adjusting the net cooling delivered. The heater adjustment can be achieved by on/off, pulse with modulated or continuously variable control means. The two methods disclosed here for modulating the net cooling delivered may be used independently or in tandem with separate PID logic loops. Schematics of three versions of the control system of this invention are provided in FIGS. 2-4.

[0038] A liquid nitrogen filling or defogging operation could cause much larger than normal vent flow rates out of the CBPU vessel. Nitrogen boil off rates increase significantly during these operations. These higher flow rates and lower vapor temperatures passing through the flow sensor could damage or negatively impact the sensor. An automatic valve could therefore be placed on exhaust line 3 between the vessel outer wall 9 and the flow sensor 2. This valve would close in response to a fill or defog signal that normally enables those operations. The valve would reopen after these operations are complete.

[0039] The modulation of net cooling delivered is preferably accomplished by direct adjustment to the active cooling system. This can be done by adjusting the electrical power supplied to cryocooler’s motor which in turn changes the driving motor’ rotary speed or stroke amplitude (depending on the type of cryocooler) and thus the net cooling produced. A second method of changing the net cooling is by thermal compensation. A heater is used that is preferably placed on the condensing surface of the cryocooler or alternately placed in the liquid nitrogen pool in the CBPU. A current is supplied to this heater to change the net cooling delivered to the CBPU while the cryocooler is running. The heater’s power may be adjusted continuously or by pulse width modulation to vary the net cooling delivered by the cryocooler. The power delivered to the cryocooler or alternately the power delivered by the compensating heater will be varied based on the output of a system controller using feedback from an instrument described below. These two modulation schemes will preferably be used independently, but may be used in tandem. The preferred type of instrument identified is a gas flow sensor that is put into a small exhaust line created into CBPU vessel through the vacuum insulated wall or through the foam insulated lid. This sensor detects a portion of the gas entering or leaving the CBPU. This sensor does not need to measure the entire flow in order to provide effective feedback to the system controller described in the next section. The instrument preferably uses thermal anemometry based technology and is able to sense both the magnitude (flow rate or velocity) and direction of flow. The flow instrument typically detects a few tens to several hundreds of cc/min of flow.

[0040] The preferred embodiment utilizes a PID programmable system controller that obtains a feedback signal from the sensor previously described. The PID controller sends an output signal which adjusts the net cooling delivered by the cryocooler through means previously described. The PID controller modulates its output signal such that the rate of cooling supplied by the cryocooler results in a vapor flow past the lid that matches pre-determined setpoint value. It is not necessary to use the integral and derivative modes of the PID controller, but these modes have been found to improve the performance of the control system in achieving and maintaining the setpoint.
0041. In light of high order system dynamics it is necessary to use conservative controller tuning to maintain closed loop stability. This constrains the performance of the closed loop control system to be relatively sluggish. It has been our observation that the feedback controller by itself is sufficient to regulate the net loss rate in the low frequency range (i.e. over long time periods); however, the aforementioned disturbances can cause short term variations that make it possible to draw air into the CBPU. One possible solution is to increase the pressure setpoint of the feedback controller. This ensures that the flow of gas is always in the desired direction; however, it increases the net loss rate. Another solution would be to use a more advanced form of feedback control (e.g. fuzzy logic or gain scheduling) to provide nonlinear control action. The controller could be programmed to be more aggressive at lower rates of flow, cutting the rate of cooling faster than the rate at which it is increased.

0042. An alternate method of net cooling modulation involves the use of a heater to provide heat to the cryocooler cold heat exchanger or to the inner space of the CBPU vessel. This heater may alternately be positioned in the vapor space of the vessel, immersed in the liquid cryogen contained in the vessel or attached to an inner wall of the vessel; this heater will preferably be an electric heater but it may alternately be a warmed fluid heat exchanger that is in a heat exchange relationship with the cryocooler cold heat exchanger or the inner space of the CBPU vessel. This alternate thermal compensation scheme is shown in FIG. 3. FIG. 4 shows an example of how the preferred and alternate cooling modulation schemes could be used in tandem. In the arrangement shown in FIG. 4, the heater is used to make quick adjustments to the net refrigeration delivered to the CBPU. PID Control Algorithm 1 compares the flow from the CBPU against a setpoint and makes quick changes to the heater power. A second controller is used, not to control the flow past the lid of the CBPU, but to try to drive the heater power to zero (thus optimizing the power consumption of the system by minimizing the power input to the heater). PID Control Algorithm 2 is a slower controller that compares the heater power against a pre-determined heater power setpoint (equal to zero) and outputs a power signal to the cryocooler based on the difference. If there is any heat being added to the CBPU by the heater, the PID Control Algorithm 2 will reduce the power supplied to the cryocooler until the heater is completely off.

0043. Although the invention has been described in detail with reference to certain preferred embodiments, those skilled in the art will recognize that there are other embodiments of the invention within the spirit and the scope of the claims. For example, the control feedback instrument may alternately be a differential pressure sensor. This sensor would not require an actual flow to be measured but it would measure the driving force required to drive a flow into or out of the vessel. Also, a fuzzy logic system controller may be used instead of a PID controller. A simpler on/off control mechanism may also be used if only very coarse control is required.

1. An actively cooled cryogenic biological preservation unit comprising:
   (A) an insulated vessel having a vessel interior, and a cryocooler positioned to provide refrigeration to the insulated container;
   (B) an exhaust line in communication with the vessel interior, and a flow sensor on the exhaust line; and
   (C) a controller, means for passing a signal from the flow sensor to the controller, and means for passing a signal from the controller to the cryocooler.

2. The actively cooled cryogenic biological preservation unit of claim 1 wherein the cryocooler is a pulse tube refrigerator.

3. The actively cooled cryogenic biological preservation unit of claim 1 wherein the controller is a proportional integral derivative controller.

4. The actively cooled cryogenic biological preservation unit of claim 1 containing at least one biological sample within the vessel interior.

5. The actively cooled cryogenic biological preservation unit of claim 1 wherein the controller is a proportional integral derivative controller.

6. The actively cooled cryogenic biological preservation unit of claim 5 further comprising a second controller, and wherein the means for passing a signal from the controller to the cryocooler includes the second controller.

7. A method for operating an actively cooled cryogenic biological preservation unit comprising:
   (A) providing refrigeration to the interior of an insulated vessel having an exhaust line in communication with the vessel interior;
   (B) sensing the flow of gas within the exhaust line; and
   (C) adjusting the amount of refrigeration provided to the vessel interior based on the gas flow sensing.

8. The method of claim 7 wherein refrigeration is provided to the vessel interior from a cryocooler.

9. The method of claim 7 wherein the gas flow sensing senses gas flowing away from the vessel interior, and the refrigeration adjustment comprises increasing the amount of refrigeration provided to the vessel interior.

10. The method of claim 7 wherein the gas flow sensing senses gas flowing toward the vessel interior, and the refrigeration adjustment comprises decreasing the amount of refrigeration provided to the vessel interior.

11. The method of claim 7 wherein the gas flow sensing senses gas flowing toward the vessel interior, and the refrigeration adjustment comprises providing heat to the vessel interior.

12. The method of claim 11 wherein the refrigeration adjustment further comprises decreasing the amount of refrigeration provided to the vessel interior.

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