Title: CRYOGENIC BIOLOGICAL PRESERVATION UNIT

Abstract: A system for the cryogenic preservation of biological material having a dedicated cryocooler (10) integrated with an insulated vessel (1) and preferably a membrane separator (33) which processes feed air (30) for supplying gaseous nitrogen (34) into the insulated vessel (1) for condensation by refrigeration provided by the dedicated cryocooler (10).
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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 140K. This trend is driven by the fact that little to no sample degradation occurs below the sample glass transition temperature which is about 140K. Conventional cryogenic biological sample preservation units that store biological samples at temperatures below 140K 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. 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.

Summary Of The Invention
[0003] One aspect of the invention is:
[0004] A cryogenic biological preservation apparatus comprising:
(A) a nitrogen source, an insulated vessel, and means for providing gaseous nitrogen from the nitrogen source into the insulated vessel; and

(B) a cryocooler mounted on the insulated vessel, said cryocooler having a cold finger positioned to provide refrigeration to the insulated vessel.

[0005] Another aspect of the invention is:

[0006] A method for operating a cryogenic biological preservation unit comprising:

(A) passing gaseous nitrogen from a nitrogen source into an insulated vessel containing at least one biological sample; and

(B) providing refrigeration from a cryocooler to the insulated vessel and condensing some of the gaseous nitrogen to form liquid nitrogen within the insulated vessel.

[0007] As used herein the term "membrane separator" means an apparatus constructed from hollow fiber tubes of membrane material that preferentially permeates oxygen over nitrogen. When pressurized feed air is passed over the tubes, a nitrogen enriched stream (retentate) as formed on the feed side, and an oxygen enriched stream (permeate) is formed inside the hollow fiber tubes.

[0008] As used herein the term "cryocooler" means a refrigerator which can produce refrigeration below 193K for the purpose of cooling biological samples.

[0009] As used herein the term "cold head" means the portion of the cryocooler containing the cold heat exchanger, aftercooler and regenerator.

[0010] 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.

[0011] As used herein the term "biological sample" means an organic material. Some examples of biological samples are proteins, blood platelets, cartilage and heart valves.

[0012] As used herein the term "feed air" means a mixture comprising primarily oxygen and nitrogen, such as ambient air.

[0013] As used herein the term "gaseous nitrogen" means a gas having a nitrogen concentration within the range of from 95 to 99.95 mole percent.

Brief Description Of The Drawings

[0014] Figure 1 is a cross sectional representation of one preferred embodiment of the cryogenic biological preservation apparatus of this invention wherein the nitrogen source is a membrane separator.

[0015] Figure 2 is a representation of a membrane separator system which may be used in the practice of the cryogenic biological preservation unit of this invention.

Detailed Description

[0016] The invention will be described in detail with reference to the Drawings. Referring now to Figure 1, there is shown a cryogenic biological preservation unit comprising an insulated vessel having a vessel wall 1 and having insulation, typically vacuum insulation, 3 adjacent the inside of vessel wall 1.
Vessel wall 1 and insulation 3 define the vessel interior or storage space 2. In the lower portion of vessel interior 2 is a pool of liquid nitrogen 4.

[0017] Within vessel interior 2 and preferably above liquid nitrogen pool 4 there is stored at least one biological sample. In Figure 1 there is illustrated in representational form a plurality of biological samples 5 on a rack system. 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.

[0018] The cryogenic biological preservation unit of this invention has an opening 20 which allows access to the vessel interior 2 from outside the vessel and through which biological samples are put into and removed from the vessel interior. Within opening 20 there is positioned lid 21 which is typically insulated using a closed cell foam such as expanded polystyrene, and which is positioned in opening 20 when access to vessel interior 2 is not desired. In the embodiment of the invention illustrated in Figure 1, lid 20 comprises a fixed portion 11 and a removable portion 7. The removable portion 7 is removed from opening 20 when access to vessel interior 2 is desired.
[0019] Any suitable cryocooler may be used in the practice of this invention. Among such cryocoolers one can name Stirling cryocoolers, Gifford-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.

[0020] 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 inerterance 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.

[0021] The cryocooler components 10 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 transferred by the oscillating working gas to the cold head 8 via the transfer tube 9. The transfer tube 9 connects the pressure wave generator to the aftercooler located at the warm end of the cold head 8, where heat is removed as previously described. The cryocooler can be controlled to provide varying amounts of refrigeration to the cold end of the cold finger 6 depending on the conditions in the cryogenic biological preservation unit vessel interior 2. This is accomplished by modulating the acoustic power output from the pressure wave generator by varying the voltage and thus the electrical power supplied. The cryocooler would preferably be controlled based on the temperature of the vessel interior 2 of the cryogenic biological preservation unit.

[0022] In the embodiment of the invention illustrated in Figure 1, cold finger 6 penetrates into vessel interior 2 and provides refrigeration directly to the vessel interior. The refrigeration cools and condenses nitrogen vapor within the upper portion of the vessel interior 2 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 4 in the lower portion of the vessel interior.

[0023] The temperature at the lowest level of the sample storage within the vessel interior may be as low as 77K and is generally within the range of from 80 to 95K. However, the normal temperature at the upper levels of the sample storage may be within the range of from 95 to 140K without the use of the integrated cryocooler 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 into the vessel. The cryocooler will also condense some gaseous
nitrogen introduced into the vessel to make up nitrogen losses. The loss rate will typically be within the range of from 20 to 400 pounds per year with the cryocooler operating.

[0024] In the embodiment of the invention illustrated in Figure 1 the nitrogen source is a membrane separator, which is the preferred nitrogen source in the practice of this invention. Other nitrogen sources, such as a nitrogen gas cylinder or liquid nitrogen container, may also be used in the practice of this invention. Referring back now to Figure 1, feed air 14, at a pressure generally within the range of from 70 to 120 pounds per square inch gauge (psig) and typically about 100 psig, and free of any moisture aerosol, is passed through supply valve 18 and as stream 30 into membrane separator system 12. Pressure relief valve 17 is used to avoid destructive overpressurization of the membrane separator. Within the membrane separator system 12 the feed air is filtered and separated into gaseous nitrogen and waste gas.

[0025] Figure 2 illustrates one preferred embodiment of a membrane separator system which may be used in the practice of this invention. The numerals in Figure 2 are the same as those of Figure 1 for the common elements. Referring now to Figure 2, the pressurized feed air 30 is fed into membrane separator system 12 which comprises prefilter 31, oil removal filter 32 and membrane separator 33. Prefilter 31 serves to remove particles as small as about 1 micron and oil removal filter 32 serves to remove oil to produce feed air which is essentially free of oil and particulate
matter. The feed air is separated in membrane separator 33 into waste gas 15 which is vented and into gaseous nitrogen which is removed from membrane separator 33 in stream 34. The gaseous nitrogen preferably has a nitrogen concentration within the range of from 99.5 to 99.95 mole percent, typically about 99.9 mole percent, and has an atmospheric dew point within the range of from -50 to -150°F, typically about -100°F.

[0026] The gaseous nitrogen is passed to control valve 13 and from control valve 13 in conduit 16 into interior space 2 of the insulated vessel. Preferably, as illustrated in Figure 1, the gaseous nitrogen is provided into the insulated vessel into the liquid nitrogen pool 4. Alternatively the gaseous nitrogen may be provided into the insulated vessel above the surface of the liquid nitrogen pool such that the stream of gaseous nitrogen impinges upon the surface of the liquid nitrogen pool.

[0027] Control valve 13 controls and meters the flow of gaseous nitrogen into the insulated vessel through inlet 16 during any desired period of time. Control valve 13 is adjusted automatically based on a signal obtained from a liquid level sensor (not shown). This control valve will have a seat orifice size to restrict the gaseous nitrogen flow into the insulated vessel sufficiently so as not to overwhelm the cryocooler and raise the storage space 2 temperature. The flow rate of gaseous nitrogen introduced into the insulated vessel through control valve 13 and inlet 16 varies, but is sufficient to maintain or build the liquid level within the vessel. Valve 18 is closed automatically.
when the membrane separator unit is not in operation and will also be used to restrict the flow of compressed feed air into the membrane separator unit as required.

[0028] The gaseous nitrogen introduced into the insulated vessel through inlet 16 is at a higher temperature than the liquid nitrogen in the vessel interior. The sensible heat of the nitrogen introduced is primarily removed by the direct heat transfer of the vapor bubbling through or impinging on the surface of the liquid nitrogen pool 4. The heating of the liquid nitrogen pool 4 causes additional saturated vapor to be produced as the liquid nitrogen pool accepts heat from the inlet gaseous nitrogen. The nitrogen vapor introduced and the additional vapor generated are then convected in the storage space and predominantly liquefied at the cold heat exchanger of the cold finger 6. The liquid generated at the cold heat exchanger is then returned by gravity to the liquid nitrogen pool 4. Any portion of the vapor that is not condensed will pass by lid 21 and vented. The cryocooler 8, 9, 10, the membrane separator 12 and control valve 13 can be controlled to build or maintain the liquid level in the insulated vessel with no external liquid source. The control mechanisms employed for these items entail sensing of the liquid level, storage space versus ambient pressure differential, and the storage space temperature, but may employ one, two or all of these sensing means.

[0029] 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 prefilter and/or the oil removal filter shown in Figure 2 need not be employed or may be employed at other locations.
CLAIMS

1. A cryogenic biological preservation apparatus comprising:
   (A) a nitrogen source, an insulated vessel, and means for providing gaseous nitrogen from the nitrogen source into the insulated vessel; and
   (B) a cryocooler mounted on the insulated vessel, said cryocooler having a cold finger positioned to provide refrigeration to the insulated vessel.

2. The apparatus of claim 1 wherein the nitrogen source is a membrane separator, and further comprising means for providing feed air to the membrane separator.

3. The apparatus of claim 2 wherein the means for providing feed air to the membrane separator comprises a prefilter and an oil removal filter.

4. The apparatus of claim 1 wherein the insulated vessel contains a pool of liquid nitrogen.

5. The apparatus of claim 4 wherein the means for providing gaseous nitrogen from the nitrogen source into the insulated vessel includes a control valve which is adjusted based on the level of the pool of liquid nitrogen within the insulated vessel.

6. The apparatus of claim 4 wherein the means for providing gaseous nitrogen from the nitrogen source into the insulated vessel provides the gaseous nitrogen into the pool of liquid nitrogen.
7. The apparatus of claim 1 wherein the insulated vessel comprises a lid and further comprises means for vapor to flow out from the insulated vessel around the lid.

8. The apparatus of claim 1 wherein the cryocooler is a pulse tube refrigerator.

9. The apparatus of claim 4 further comprising at least one biological sample above the pool of liquid nitrogen.

10. A method for operating a cryogenic biological preservation unit comprising:
    (A) passing gaseous nitrogen from a nitrogen source into an insulated vessel containing at least one biological sample; and
    (B) providing refrigeration from a cryocooler to the insulated vessel and condensing some of the gaseous nitrogen to form liquid nitrogen within the insulated vessel.

11. The method of claim 10 wherein the nitrogen source is a membrane separator, and further comprising separating feed air in the membrane separator to produce gaseous nitrogen.

12. The method of claim 10 wherein the gaseous nitrogen is passed continuously over a period of time from the nitrogen source into the insulated vessel.
13. The method of claim 12 wherein some gaseous nitrogen is passed out of the insulated vessel during the said period of time.

14. The method of claim 12 wherein the flowrate of gaseous nitrogen into the insulated vessel during the said period of time is changed at least once causing a change in the amount of liquid within the insulated vessel.

15. The method of claim 10 wherein the liquid nitrogen within the insulated vessel forms a pool and the gaseous nitrogen is passed from the nitrogen source into the pool of liquid nitrogen.

16. The method of claim 10 wherein the liquid nitrogen within the insulated vessel forms a pool having a surface, and the gaseous nitrogen is passed from the nitrogen source into the insulated vessel such that it impinges upon the surface of the pool of liquid nitrogen.