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(54) **BACTERIA-BASED GAS GENERATOR**

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(63) Continuation of application No. 13/112,368, filed on May 20, 2011, now Pat. No. 9,327,804, which is a continuation-in-part of application No. 12/700,759, filed on Feb. 5, 2010.

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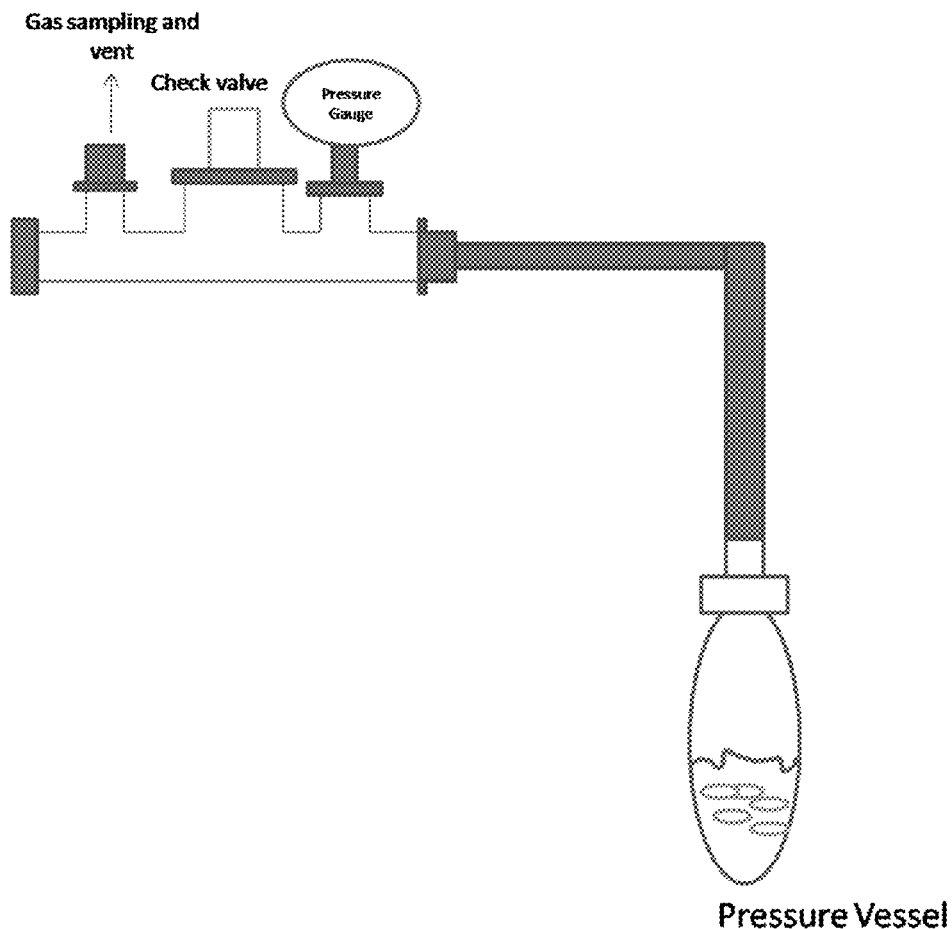
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(57) **ABSTRACT**

(22) Filed: **May 3, 2016**

Disclosed is an apparatus having: a pressure chamber and a gas-producing microorganism within the chamber. The pressure chamber is capable of maintaining a gas pressure of at least 0.5 psi above atmospheric pressure.



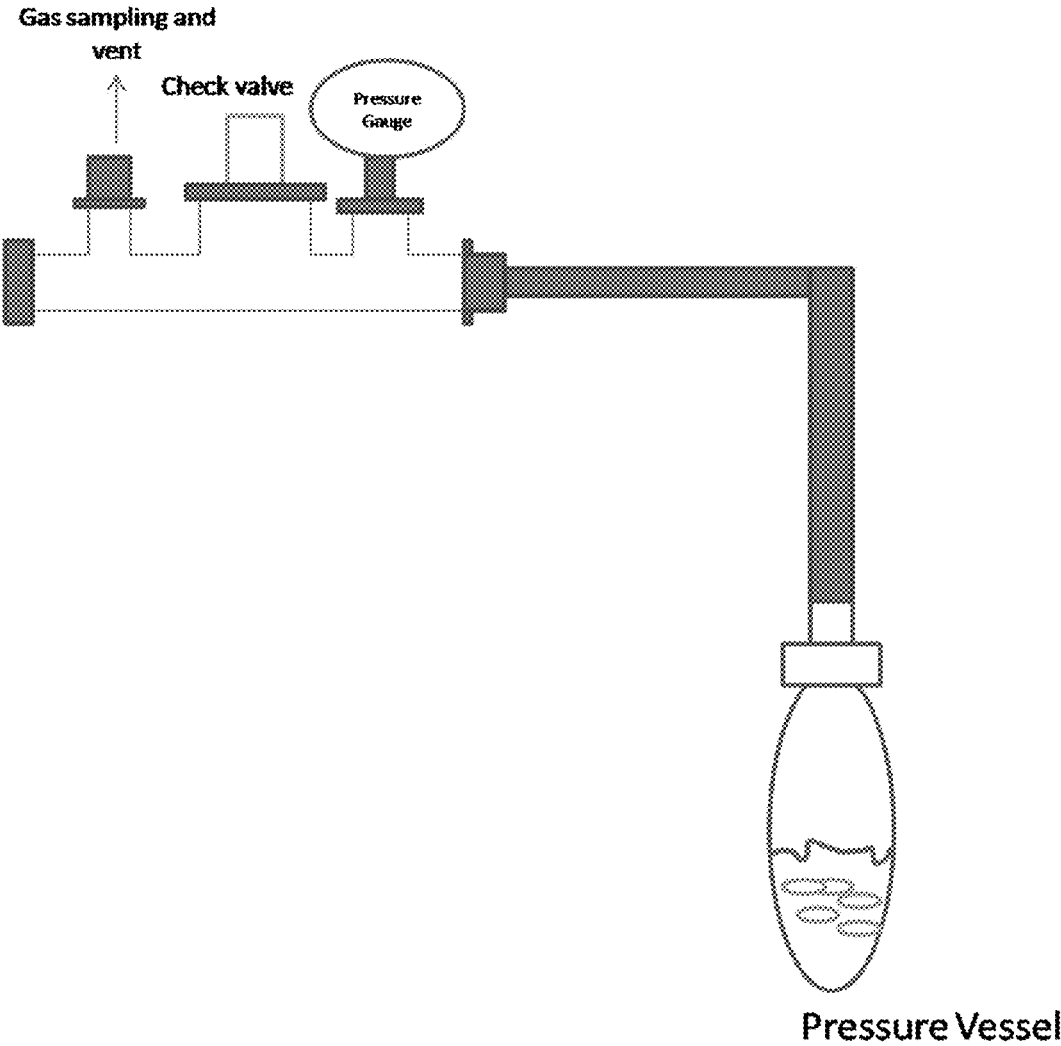


Fig. 1

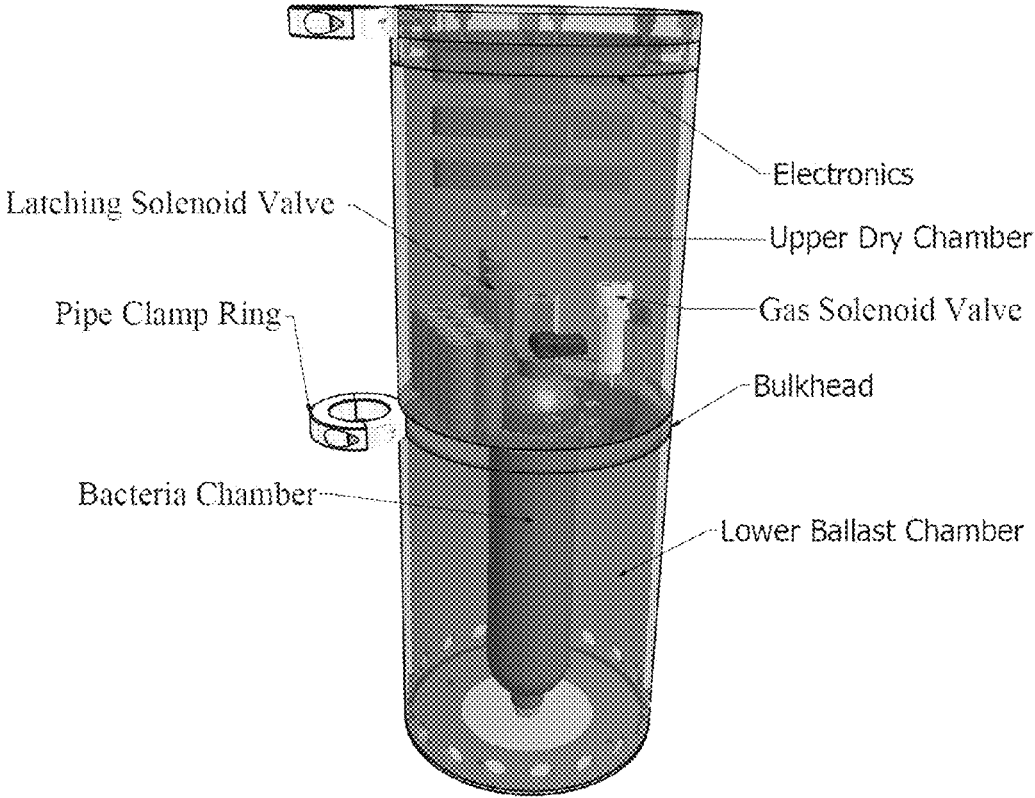


Fig. 2

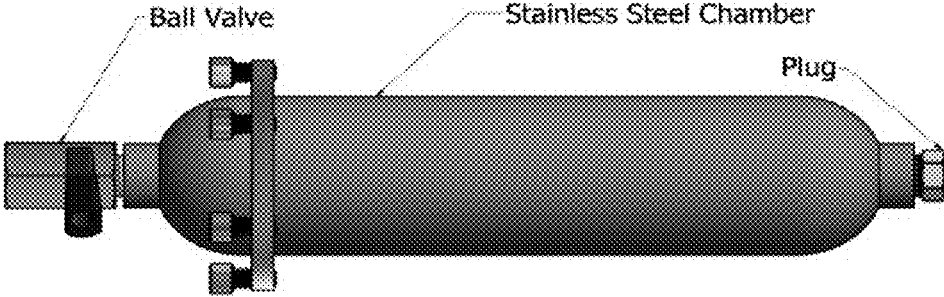


Fig. 3

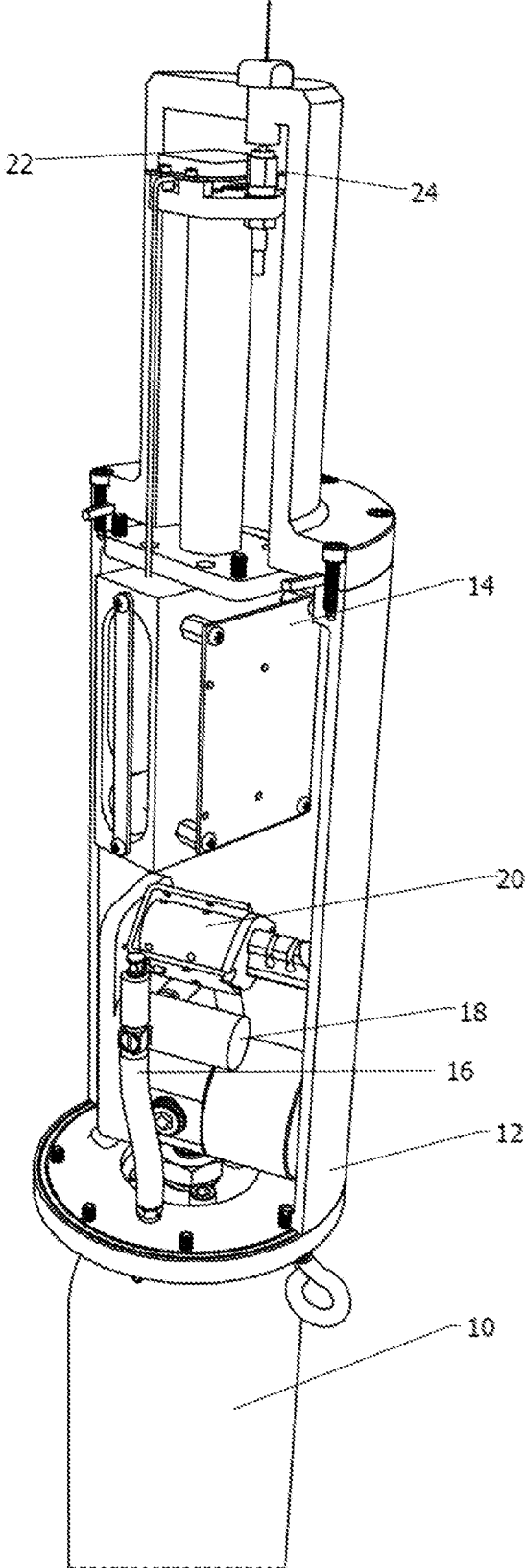


Fig. 4

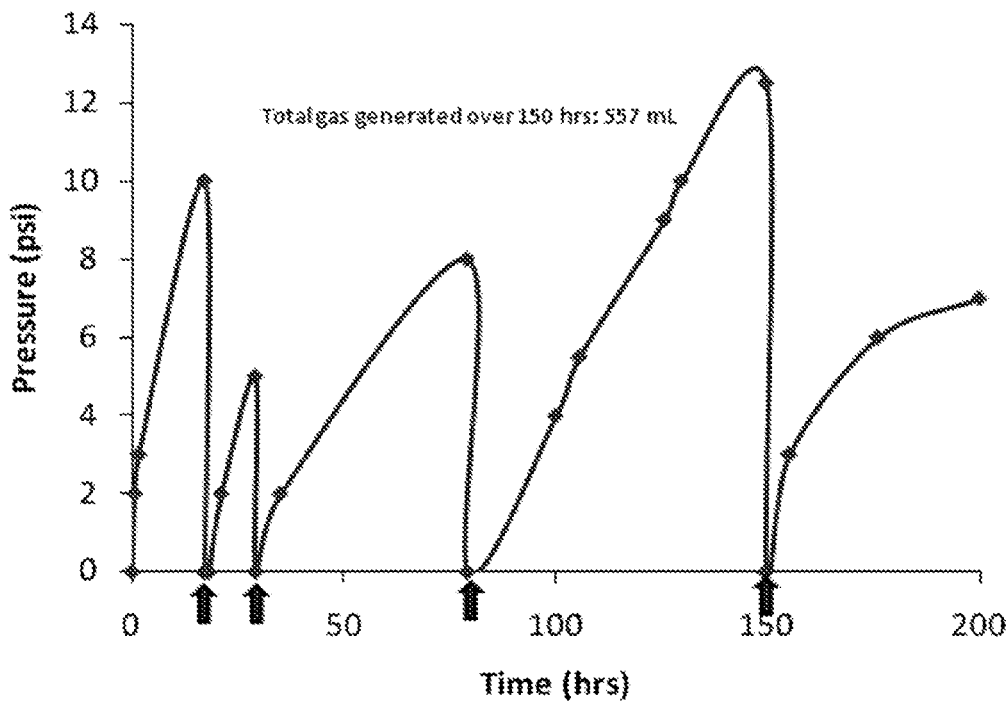


Fig. 5

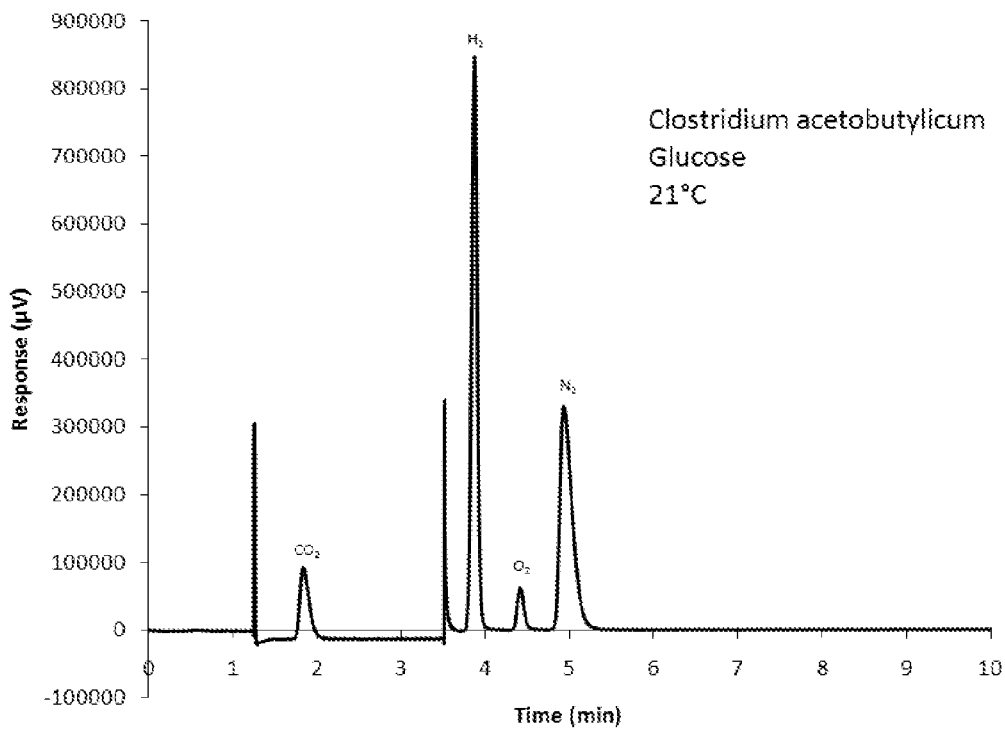


Fig. 6

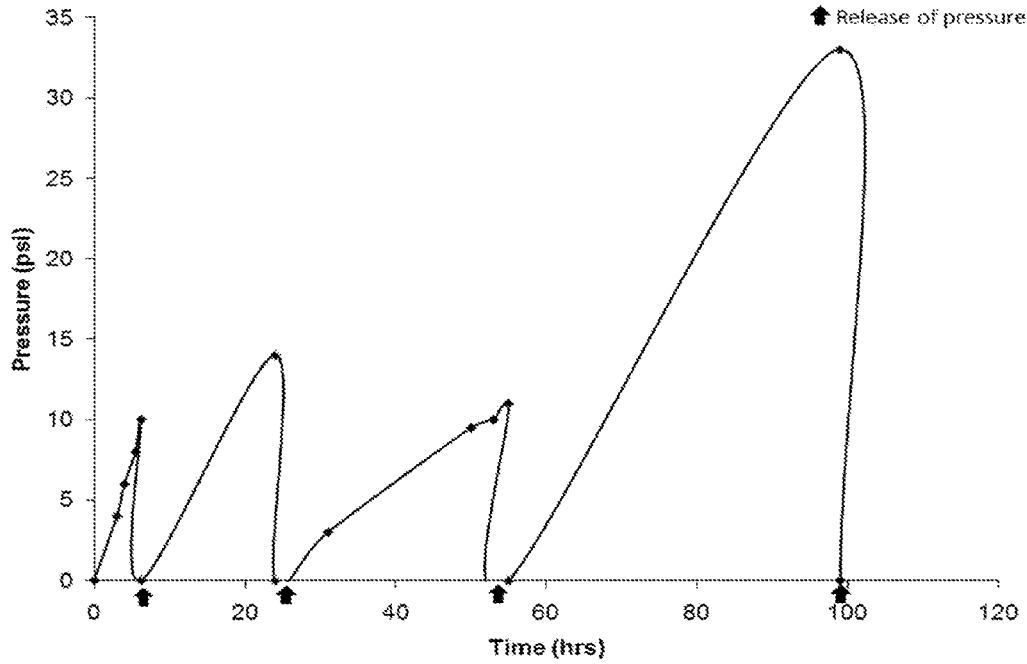


Fig. 7

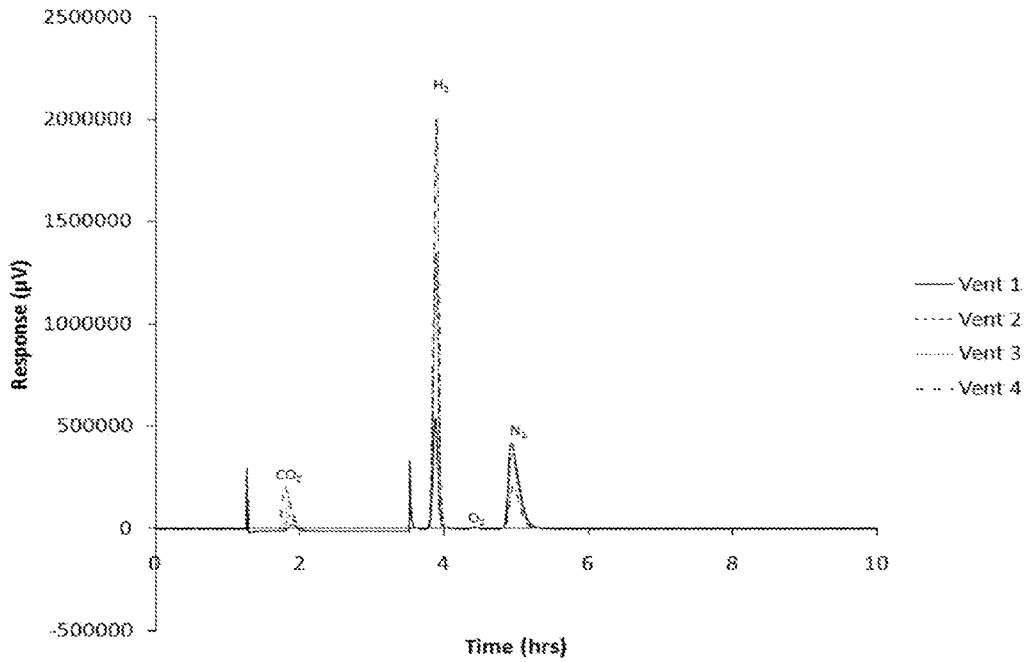


Fig. 8

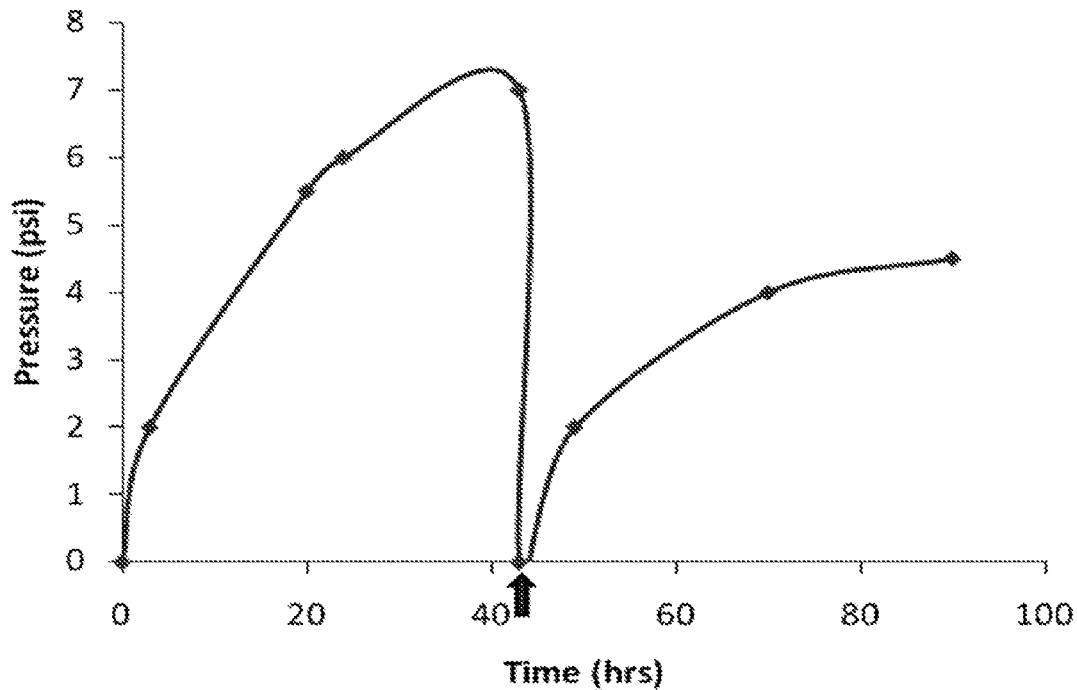


Fig. 9

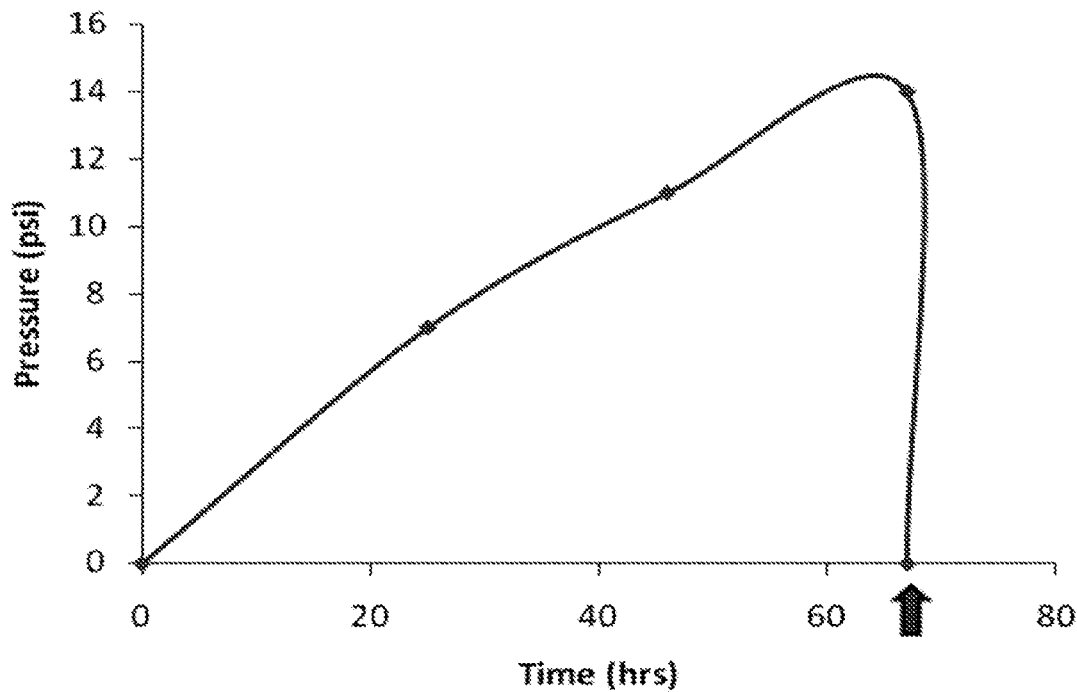


Fig. 10

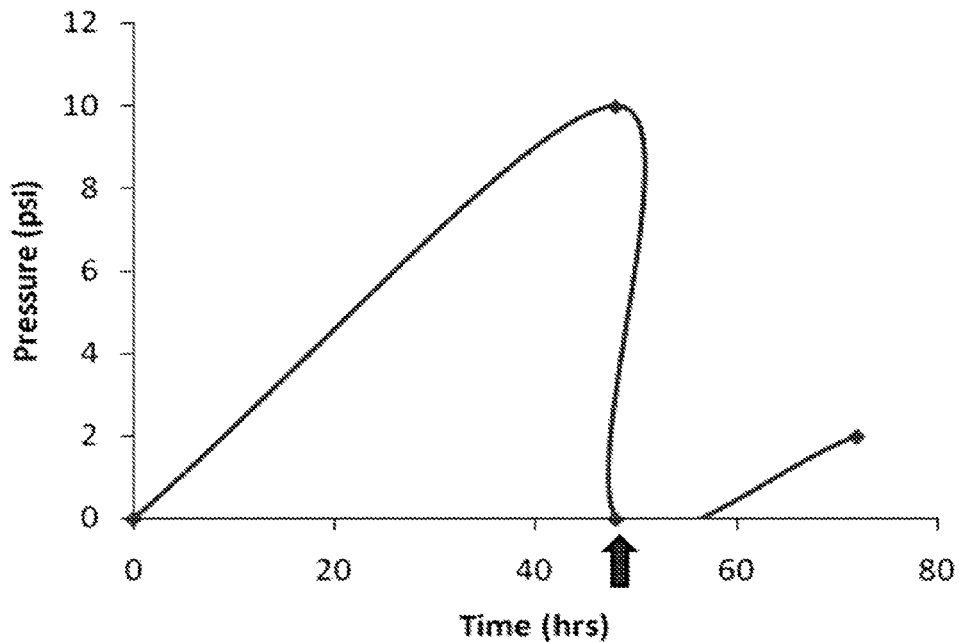


Fig. 11

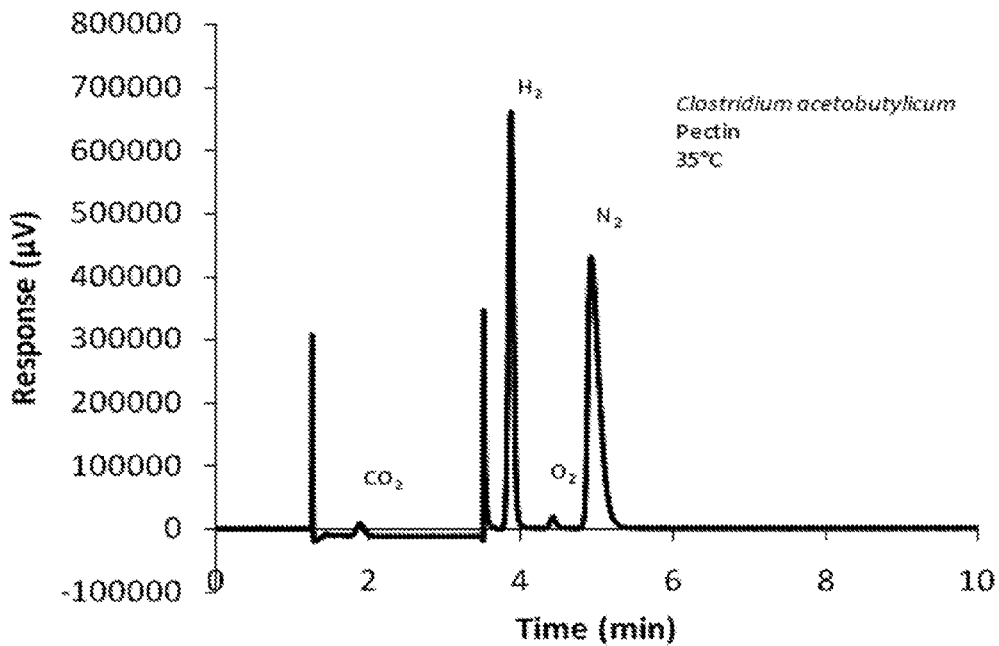


Fig. 12

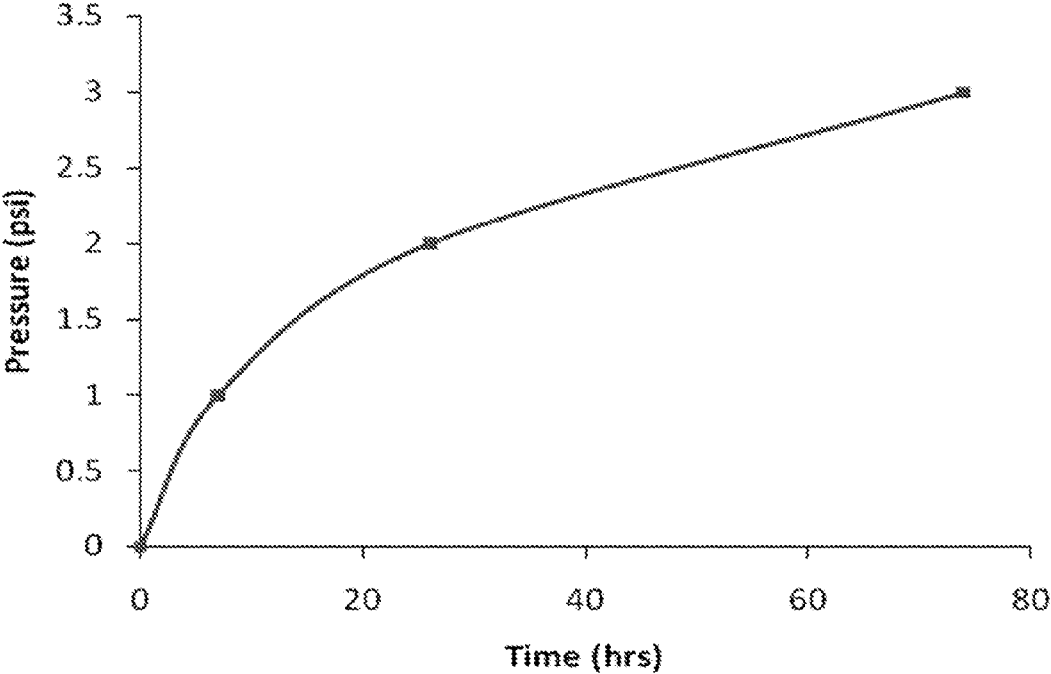


Fig. 13

BACTERIA-BASED GAS GENERATOR

[0001] This application is a continuation application of U.S. Pat. No. 9,327,804, issued on May 3, 2016, which claims the benefit of U.S. Provisional Application No. 61/346,530, filed on May 20, 2010, and is a continuation-in-part application of pending U.S. patent application Ser. No. 12/700,759, filed on Feb. 5, 2010, which claims the benefit of U.S. Provisional Application No. 61/150,446, filed on Feb. 6, 2009. These applications and all other publications and patent documents referred to throughout this nonprovisional application are incorporated herein by reference.

TECHNICAL FIELD

[0002] The present disclosure is generally related to the generation and containment of gas from microorganisms.

BRIEF SUMMARY

[0003] Disclosed herein is an apparatus comprising: a pressure chamber and a gas-producing microorganism within the chamber. The pressure chamber is capable of maintaining a gas pressure of at least 0.5 psi above atmospheric pressure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] A more complete appreciation of the invention will be readily obtained by reference to the following Description of the Example Embodiments and the accompanying drawings.

[0005] FIG. 1 shows a general schematic for a pressure chamber.

[0006] FIG. 2 shows a general schematic for autonomous floating system using pressurized bacterial ballast.

[0007] FIG. 3 shows a general design for bacterial gas tank used in autonomous floating system.

[0008] FIG. 4 shows a general schematic for a deployed sensor.

[0009] FIG. 5 shows pressure generated from *Clostridium acetobutylicum* using glucose (10% w/w) in a 11.2 g/L agar support over 200 hours at 21° C. Release of pressure is indicated by arrows. Tube was filled to 30% capacity.

[0010] FIG. 6 shows a GC/TCD chromatograph of gas composition generated by *Clostridium acetobutylicum* from glucose (10% w/w) at 21° C. Residual oxygen and nitrogen are from the injection.

[0011] FIG. 7 shows pressure generated from *Clostridium acetobutylicum* using glucose (10% w/w) in a 11.2 g/L agar support at 35° C. Release of pressure is indicated by arrows. Tube was filled to 30% capacity.

[0012] FIG. 8 shows a GC/TCD chromatograph of gas composition generated by *Clostridium acetobutylicum* from glucose (10% w/w) at 35° C. Residual oxygen and nitrogen are from the injection.

[0013] FIG. 9 shows pressure generated from *Clostridium acetobutylicum* using glucose (10% w/w) in a 3.8 g/L agar support at 21° C. Release of pressure is indicated by arrows. Tube was filled to 30% capacity.

[0014] FIG. 10 shows the maximum pressure generated from *Clostridium acetobutylicum* using glucose (10% w/w) in a 11.2 g/L agar support over 65 hours at 21° C. Release of pressure is indicated by arrows. Tube was filled to 50% capacity.

[0015] FIG. 11 shows pressure generated from *Clostridium acetobutylicum* using pectin (5% w/w) in a 11.2 g/L agar

support at 35° C. Release of pressure is indicated by arrows. Tube was filled to 50% capacity.

[0016] FIG. 12 shows a GC/TCD chromatograph of gas composition generated by *Clostridium acetobutylicum* from pectin (5% w/w) at 35° C. Residual oxygen and nitrogen are from the injection.

[0017] FIG. 13 shows pressure generated from *Clostridium acetobutylicum* using pectin (5% w/w) in a 11.2 g/L agar support at 21° C. Release of pressure is indicated by arrows. Tube was filled to 50% capacity.

DETAILED DESCRIPTION OF EXAMPLE EMBODIMENTS

[0018] In the following description, for purposes of explanation and not limitation, specific details are set forth in order to provide a thorough understanding of the present disclosure. However, it will be apparent to one skilled in the art that the present subject matter may be practiced in other embodiments that depart from these specific details. In other instances, detailed descriptions of well-known methods and devices are omitted so as to not obscure the present disclosure with unnecessary detail.

[0019] Disclosed herein is an apparatus and methods for repeatedly generating gas pressure from microorganisms and bacteria to greater than 30 psi. The gas may optionally be primarily composed of hydrogen, which is considered a potential large scale fuel source of the future (Misra et al., "Hydrogen Evolution on Hydrophobic Aligned Carbon Nanotube Arrays" *ACS Nano* 2009, 3(12), 3903-3908; Ye et al., "Hydrogen generation utilizing alkaline sodium borohydride solution and supported cobalt catalyst" *Journal of Power Sources* 2007, 164(2), 544-548). The apparatus may be used with *Clostridium acetobutylicum*, a bacterium known for producing hydrogen from carbohydrate sources (Alshiyab et al., "Improvement of biohydrogen production under increased the reactor size by *C. acetobutylicum* NCIMB 13357" *American Journal of Environmental Sciences* 2009, 5(1), 33-40; Zhang et al., "Biohydrogen production in a granular activated carbon anaerobic fluidized bed reactor" *International Journal of Hydrogen Energy* 2007, 32(2), 185-191). This system is not limited to solely *Clostridium* spp., but encompasses any microorganism capable of generating gas at rates that produce significant pressure from renewable biomass.

[0020] The system may extend operational durations of autonomous sensors or unmanned underwater vehicles (UUV) and also could be used for portable power supplies. Re-pressurizing gas tanks with bacteria may eliminate the need to transport tanks to external fueling stations, generate gaseous fuels on-site using biomass, and provide ballast for long-duration aquatic sensors, communication relays, and buoys. Generating pressure via bacteria may enable the production of fuels and ballast separate from fossil fuel derived methods and processes.

[0021] FIG. 1 shows a general schematic for a pressure chamber. The apparatus and method are based on the goal of generating pressure using bacterial gas production within a single chamber to greater than, for example, 0.5, 3, 10, or 30 psi above standard atmospheric pressure or higher. The pressure chamber may be any container generally used to contain a gas or other container that does not deform, rupture, or otherwise change its interior volume at pressures up to the operational gas pressure. The chamber could be fabricated

out of glass, as well as standard non-corrosive alloys used in commercial gas tank operations

[0022] The produced gas may be, for example, hydrogen, carbon dioxide, or energetic gases or gaseous fuels such as hydrocarbon gases, methane, ethane, and propane. The gas may be produced in anaerobic or aerobic conditions depending on the microorganism and the gas. The gas may be produced by a fermentative process.

[0023] Any microorganism that produces a gas may be used. Suitable hydrogen-producing microorganisms include, but are not limited to, *Clostridium* sp., *Clostridium acetobutylicum*, *Escherichia coli*, *Clostridium thermocellum*, *Clostridium cellulolyticum*, *Clostridium butyricum*, *Enterobacter aerogenes*, *Desulfovibrio vulgaris*, *Magashaera elsdeni*, *Citrobacter intermedius*, *Enterobacter cloacae*, and *Clostridium pasteurianum*. Energetic gases may be produced by, for example, *Methanococcus bourgensis*, *Methanomicrobiales* sp., *Methanobacterium* sp., *Methanobacterium bryantii*, *Methanobacterium formicum*, *Methanopyrus kandleri*, *Methanococcus* sp., *Methanococcus jannaschii*, *Methanococcus deltae*, *Methanococcus maripaludis*, *Methanococcus vanniellii*, and *Methanococcus marisnigri*. These methane producing bacteria may also have the potential to produce smaller quantities of ethane and propane. An agar matrix may act as a growth support for the stabilization of gas generation.

[0024] The pressure chamber may be coupled to a vent that may release the gas produced by the microorganism. The vent may be an integral part of the pressure chamber or may be coupled to the pressure chamber through one or more intermediate parts, such as tubing. Any form of vent may be used. The vent may automatically release gas when the pressure inside the chamber reaches a threshold level, or may release gas periodically through the use of a timer. The vent may also be opened manually or robotically at any time or based on any criteria.

[0025] When a food source appropriate for the microorganism and any desired gas are within the pressure chamber such that the microorganism can metabolize the food, the resulting gas may be allowed to build up over time to an elevated pressure. The pressure may build up to, for example, 0.5, 5, 10, or 30 psi above standard atmospheric pressure or higher. The built-up pressure or portion thereof may then be vented through the vent. The venting may be triggered by the level of the pressure in the chamber or by other criteria. The pressure may be vented back to atmospheric pressure or maintained at a higher level.

[0026] The process of building up gas pressure and venting the gas may be repeated one more times. This may be continued potentially until the food source is no longer sufficient to increase the pressure. The process may continue for longer or even indefinitely by placing additional food source in the chamber. The food source may be added in batches or continuously. The food may be added through an optional food inlet.

[0027] Additional components may be incorporated with the apparatus to exploit the venting of the gas from the chamber. In one embodiment the pressure chamber is enclosed in a container having a divider or bulkhead that divides the container into a ballast chamber and a dry chamber. The vent can move gas from the pressure chamber to the dry chamber, a gas valve moves gas from the dry chamber to the ballast chamber, and a ballast valve moves liquid between the ballast chamber and the exterior of the container.

[0028] When the container is submerged in a liquid, such as water or ocean water, gas may be vented into the dry chamber and subsequently into the ballast chamber. This displaces water in the ballast chamber to the exterior, resulting in the container rising in the liquid. When the ballast container is refilled with liquid, the container sinks.

[0029] FIG. 2 schematically illustrates an example of this embodiment. The floater device has the objective of floating and sinking itself in a marine environment by utilizing gas producing bacteria. Design considerations for the device include:

[0030] 1. The device should automatically rise to the surface along a tether, pause at the top and then sink again. It should be able to perform this several times per day

[0031] 2. The device should resist corrosion, clogging, and the pressure of 10 meters of sea water. It should not sink into the sand or sediment of the ocean floor.

[0032] 3. The gas produced by the bacteria is hydrogen. Special material will need to be utilized to minimize the leakage of hydrogen.

[0033] 4. The bacterial chamber should be filled with agar in an anaerobic environment. The chamber should also permit the injection of the bacteria into the agar with a syringe and needle. The injection should be possible in the field while maintaining the anaerobic environment.

[0034] 5. The valve system should use minimal power, permitting reduced battery size and increased operating time.

[0035] The example floater device (FIG. 2) is a cylinder consisting of an upper dry chamber and lower ballast chamber. The upper dry chamber contains the electronics, batteries, a gas solenoid valve, a latching solenoid valve, and a pressure data recorder. The bacterial chamber is screwed in between the two chambers and sealed with an o-ring. There are multiple fittings on the bulkhead between the two chambers that accommodates gas lines and a pressure sensor to determine the ocean depth of the floater.

[0036] The gas solenoid valve controls the flow of hydrogen into the ballast tank. The latching solenoid valve vents the ballast tank to the outside environment. This latching solenoid valve is a "pinch" type. The "pinch" type valve permits the only whetted material in the valve to be the tube. The tube may be PharMed® tubing, which holds up very well to the ocean environment.

[0037] While resting on the ocean floor, the latching solenoid valve remains in its open position. To rise, the system closes the latching valve, sealing off the ballast tank. The gas valve then briefly opens to fill the ballast tank. With its increased buoyancy, the floater rises to the surface. After a period of time at the surface, the latching solenoid valve returns to its venting state and the floater begins its decent along the tether. Two pipe clamp rings are attached to the side of the floater to guide it up and down the tether.

[0038] The bacteria chamber is stainless steel and has metal fittings at each end to prevent hydrogen leakage. The top fitting is a nickel-coated brass ball valve which permits the chamber to be filled with agar in an inert environment and then sealed off for transport. This ball valve is fitted with a HPLC fitting to mate with the bacteria tank valve.

[0039] The bottom of the bacterial chamber is fitted with a stainless steel plug (FIGS. 2-3). When the agar is cast, it forms against this plug. In the field, the plug is removed and bacteria are injected directly into the agar. Forming the agar against the plug creates virtually no head space between the two.

When the bacteria are injected and the plug replaced, virtually no oxygen is introduced into the chamber.

[0040] In another embodiment, the apparatus is the source of gas for use in the buoyancy control device disclosed in US Patent Application Publication No. 2010/0199907. This device has a floater chamber having a gas inlet coupled to the vent, a second gas vent, and a liquid vent; and a float and a weight coupled to the floater chamber. The float has a lower density than the floater chamber. The weight has a higher density than the floater chamber. The aggregate density of the floater chamber, the float, and the weight is greater than the density of the floater chamber. The gas inlet, the second gas vent, the liquid vent, the float, and the weight are positioned on the floater chamber such that when the floater chamber is filled with and submerged in a liquid in which the floater chamber is neutrally-buoyant, the floater chamber is oriented to place the second gas vent below the gas inlet; and when a gas is introduced through the gas inlet into the floater chamber that is filled with the liquid, the floater chamber pivots to raise the second gas vent until a portion of the gas escapes from the floater chamber through only the second gas vent.

[0041] When a portion of the gas is vented into the floater chamber, the floater chamber pivots until a portion of the gas escapes from the floater chamber through the second gas vent. After enough gas escapes, the floater chamber returns to a position at which gas does not escape from the floater chamber. This process can be repeated. Additional details may be found in US Patent Application Publication No. 2010/0199907.

[0042] An another embodiment, an inflatable bladder have a release vent is coupled to the vent. Venting gas into the bladder causes the device to rise in the liquid. Venting the bladder to the exterior causes the device to sink. In one example (FIG. 4) a pressure vessel **10** is attached to a secondary compartment **12** with all electronics **14** necessary for a deployed sensor. There is a regulator **16** attached directly to the pressure vessel **10** so that no matter what the pressure is inside the vessel, the output pressure is regulated to be less than 80 psi. There is a valve **18** attached to the regulator **16** that will release a portion of the pressure to blow up a bladder (causing a positive buoyant force) and another valve **20** attached to the bladder to release the pressure and deflate at a later time. The

bladder (not shown) surrounds the electronics container. This container may contain a GPS **22**, a microchip (not shown) to process acoustic data/algorithms and to control the surfacing/sinking valves, and radio transmission **24** to a satellite.

[0043] Hydrogen generation was used for the purpose of parallel fuel synthesis in the same system. Two different agar concentrations were used for proof of principle (11.2 g/L agar (75%) and 3.8 g/L agar (25%)).

[0044] The following examples are given to illustrate specific applications. These specific examples are not intended to limit the scope of the disclosure in this application.

EXAMPLE 1

[0045] The results from the two different agar concentrations confirmed that matrices containing more liquid are less supportive of elevated pressures. An agar concentration of 11.2 g/L generated the highest pressure and, as long as agar concentration does not begin to hinder gas production or bacterial growth, higher concentrations of agar should generate higher pressures. The general experimental details are below. Pressure measurements were performed in a glass system purchased from Ace glass. The general schematic of the system is shown in FIG. 1.

[0046] Agar at 11.2 g/L concentration (75%) in Reinforced Clostridial Medium amended with 10% w/w glucose was used as the base support for growth of *Clostridium acetobutylicum* (ATCC 39236) under an inert atmosphere. In 100 mL glass 1 neck pressure tubes, 30 mL of the sterilized hot agar medium was filled. The sterilized medium was allowed to degas and cool in the anaerobic chamber. Each tube was inoculated with active *C. acetobutylicum* (determined by the formation of gas bubbles) by stabbing into the agar supports 5-7 times. Each tube was then sealed with gas tight pressure seals and connected to an analog pressure gauge. Time and pressure measurements were recorded manually. The experiment was performed at 21° C. and 35° C. (FIGS. 5-13, Tables 1-3).

[0047] The pressure that was generated reached a maximum pressure over 24 hours. The system was vented and the gas that was generated was analyzed using a Varian gas chromatograph with thermal conductivity detector (GC/TCD) calibrated for CO₂, CO, H₂, CH₄, O₂, and N₂ with a custom valve setup and packed columns (FIGS. 5-13, Tables 1-3).

TABLE 1

Analytical data from GC/TCD experiment in FIG. 6						
Index	Name	Time (min)	Quantity (%)	Height (μV)	Area (μV · min)	Area %
1	CO ₂	1.86	5.03	75908.4	9457.2	5.944
2	hydrogen	3.88	61.42	1050319.2	83345.5	52.381
3	unknown	4.16	0.00	1860.7	139.6	0.088
4	oxygen	4.42	4.01	92894.2	9091.9	5.714
5	nitrogen	4.94	29.54	330127.0	57032.2	35.843
6	unknown	10.00	0.00	1006.3	47.9	0.030
Total			100.00	1552115.8	159114.4	100.000

TABLE 2

Analytical data from GC/TCD experiment in FIG. 8						
Index Name	Time (min)	Quantity (%)	Height (μV)	Area ($\mu\text{V} \cdot \text{min}$)	Area %	
Vent 1						
1	CO ₂	1.89	2.73	75908.4	9457.2	5.944
2	hydrogen	3.87	43.44	1050319.2	83345.5	52.381
3	oxygen	4.42	4.01	92894.2	9091.9	5.714
4	nitrogen	4.93	29.54	330127.0	57032.2	35.843
Total		100.00	1552115.8	159114.4		100.000
Vent 2						
1	CO ₂	1.86	4.18	75195.4	9240.3	5.102
2	hydrogen	3.88	67.82	1344784.0	108211.3	59.753
3	oxygen	4.42	0.22	4890.4	577.0	0.319
4	nitrogen	4.94	27.78	364734.7	63069.4	34.826
Total		100.00	1789604.0	181098.0		100.000
Vent 3						
1	CO ₂	1.84	7.40	124815.8	16038.1	9.021
2	hydrogen	3.88	67.25	1314890.0	105227.0	59.185
3	oxygen	4.42	0.25	5567.7	657.0	0.370
4	nitrogen	4.94	25.10	322473.3	55872.4	31.425
Total		100.00	1767747.0	177794.5		100.000

TABLE 3

Analytical data from GC/TCD experiment in FIG. 12						
Index Name	Time (min)	Quantity (%)	Height (μV)	Area ($\mu\text{V} \cdot \text{min}$)	Area %	
1	CO ₂	1.89	1.53	19816.1	2291.5	1.734
2	hydrogen	3.87	48.40	662526.2	52400.1	39.659
3	oxygen	4.42	1.03	18273.9	1865.7	1.412
4	unknown	4.65	0.00	646.2	17.6	0.013
5	nitrogen	4.93	49.04	431644.4	75539.0	57.172
6	unknown	7.95	0.00	284.6	11.5	0.009
Total		100.00	1133191.4	132125.4		100.000

EXAMPLE 2

[0048] Due to the possibility of bacterial death due to exposure of the bacterial cells to high levels of pressure (thereby creating an unsustainable ballast system), *C. acetobutylicum* cell viability was tested under varying pressures. *C. acetobutylicum* cells were grown in Reinforced Clostridial Medium at an 11.2 g/L agar concentration amended with 10% w/w glucose. Cells were then subjected to different pressures (0, 5, 10, 20, and 30 psi) and stained with a live/dead bacterial stain (Invitrogen LIVE/DEAD BacLight kit) to determine the viability of cells following exposure to pressures. Showing the live/dead stain works properly, cells intentionally killed in 30% H₂O₂ were stained and found to be 100% dead, while cells at 0 psi (presumed to be mostly live) were stained and indeed found to be alive at a reasonable proportion to dead cells. The other pressures (5, 10, 20, 30 psi) showed the same proportion of live/dead cells as at 0 psi indicating that pressure does not decrease cell viability and that the ballast system is indeed sustainable.

[0049] Obviously, many modifications and variations are possible in light of the above teachings. It is therefore to be understood that the claimed subject matter may be practiced otherwise than as specifically described. Any reference to

claim elements in the singular, e.g., using the articles “a,” “an,” “the,” or “said” is not construed as limiting the element to the singular.

What is claimed is:

1. An apparatus comprising:
 - a pressure chamber;
 - a gas-producing microorganism within the chamber;
 - wherein the pressure chamber is capable of maintaining a gas pressure of at least 0.5 psi above atmospheric pressure; and
 - a vent coupled to the pressure chamber configurable to allow the gas pressure in the chamber to increase and configurable to release gas produced by the microorganism.
2. The apparatus of claim 1, wherein the microorganism is a fermentative gas-producing microorganism.
3. The apparatus of claim 1, wherein the microorganism is a hydrogen-, methane- or carbon dioxide-producing microorganism.
4. The apparatus of claim 1, wherein the microorganism is *Clostridium* sp. or *Clostridium acetobutylicum*.

5. A method comprising:
providing the apparatus of claim 1;
placing a food source for the microorganism within the pressure chamber; and
waiting for the gas pressure in the pressure chamber to increase to at least 0.5 psi above atmospheric pressure.
6. The method of claim 5, further comprising:
venting a portion of the gas through the vent.
7. The method of claim 6, further comprising:
repeating waiting for the gas pressure to increase and venting a portion of the gas one or more times.
8. A method comprising providing the apparatus of claim 1;
wherein the pressure chamber is capable of maintaining a gas pressure of at least 10 psi above atmospheric pressure;
placing a food source for the microorganism within the pressure chamber; and
waiting for the gas pressure in the pressure chamber to increase to at least 10 psi above atmospheric pressure.
9. The method of claim 8, further comprising:
venting a portion of the gas through the vent.
10. The method of claim 9, further comprising:
repeating waiting for the gas pressure to increase and venting a portion of the gas one or more times.

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