



(51) International Patent Classification:
H01M 8/16 (2006.01)

(21) International Application Number:
PCT/US2020/015478

(22) International Filing Date:
28 January 2020 (28.01.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
16/259,875 28 January 2019 (28.01.2019) US

(72) Inventor; and

(71) Applicant: **BITTERLY, Steve** [US/US]; 8900 Eton Avenue, Suite #F, Canoga Park, California 91304 (US).

(72) Inventor: **BITTERLY, Jack** (deceased).

(74) Agent: **HARRIS, Michael D.** et al.; SoCal IP Law Group LLP, 310 N Westlake Blvd., Suite 120, Westlake Village, California 91362 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

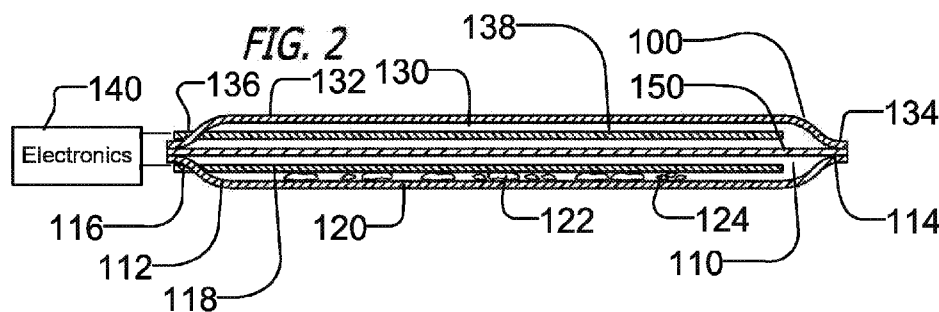
SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) Title: SENSOR FOR DETECTING MICROORGANISMS AND CORRESPONDING PROCESS



(57) Abstract: Microbial fuel cells generate an electrical signal when microbes enter the cells through a semipermeable membrane. The single chamber microbial fuel cell comprises an anode adapted to be positioned near a potentially contaminated source to receive microbes on the source, a cathode spaced from the anode and carbohydrate positioned between the anode and cathode. The anode is flexible to adapt to a source that might have microbes. When microbes enter the fuel cell and reach the anode, the fuel cell generates an electrical signal. By reading and analyzing the signal from one or more of the fuel cell biosensors can indicate infection in people or animals, indicate pathogens growing in food or show mold growth. Insofar as different microbes have specific metabolisms, the signal may be used to determine which microbe is present.



SENSOR FOR DETECTING MICROORGANISMS AND CORRESPONDING PROCESS

BACKGROUND

[0001] *1. Field:* A sensor and corresponding process detects microorganisms such as bacteria, molds or viruses and identifies the microorganism.

[0002] *2. Related Application:* This application claims priority to provisional application No. 61/450,342, filed March 8, 2011, by Jack G. Bitterly and Steve E. Bitterly, to non-provisional application No. 13/415,702, filed March 8, 2012, by Jack G. Bitterly and Steve E. Bitterly.

[0003] *3. General Background and State of the Art:* Identifying whether a particular pathogenic microorganism is present can be crucial for human or animal patients and for other applications.

[0004] One way to determine the presence of bacteria on or in a patient is through culturing a sample. Though techniques for aerobic and anaerobic bacteria are different, they are well known. If a physician suspects, for example, that a child has strep throat, a Group A streptococci infection, the physician or nurse takes a mucus sample from the child's throat with a swab and rubs the swab onto agar in a Petri dish. There, any bacteria, if present, incubate. After several days, a technician can determine if streptococci are present. One can also culture microorganism from different sites such as around a wound or surgery or in the blood. Similar incubation techniques also work for molds although the culturing techniques may differ. Some bacteria and many molds also resist culturing. But culturing on a medium in a Petri dish is an accepted practice.

[0005] This culturing procedure takes time and is expensive. Physicians and veterinarians usually want to know immediately whether to begin an antibiotic regimen. They face pressure to prescribe antibiotics if they suspect a bacterial infection. Standard protocol following some surgeries requires administering antibiotics even without symptoms of an infection. However, many believe that overuse of antibiotics is a serious problem that has caused antibiotic resistant strains by many bacteria. A physician confident that the patient or wound is free from infection might avoid using antibiotics. If infection has begun, however, the physician can be confident that the patient needs antibiotics.

[0006] Bacteria multiply rapidly, exponentially under ideal conditions. When relatively few bacteria are present, the patient may exhibit no symptoms of an infection, but culturing samples may be hit or miss. A sample from part of a wound may yield bacteria; another part of the wound may yield none.

[0007] Delay seems built into culturing. If the physician does not begin treatment until he or she receives positive results, the infection may become serious. Similarly, one sample may find one strain of bacteria, but may overlook a more serious, virulent pathogen. Antibiotics administered for the bacteria found in the sample may not be ideal for other strains in adjacent areas. Delaying treatment may allow an infection to grow and cause severe illness or death.

[0008] Hospital- or office-based physicians rarely perform culturing themselves. In an office practice, the physician sends the sample to an outside laboratory. The transportation of the sample causes delay. Delay also occurs as the lab performs the tests and then reports to the physician. Even hospitals, which may have their own on-site labs, sample transportation to the lab and delay in transmitting results are usual. For hospitals that subcontract lab services to off-site labs, the delay can increase.

[0009] Bacteria also contaminate food. For example, according to the United States Centers for Disease Control (CDC), over 70,000 Americans are infected annually by *E coli* O157:H7, usually from contaminated ground beef. Over 60 of the infected die, and others suffer severe symptoms. People also have been sickened or died from eating infected milk, cheese and chicken. The United States government estimates that food-borne diseases cause over 80 million illnesses, 350,000 hospitalizations, 5,000 deaths, and US\$3.1 billion in costs yearly. Meat spoilage due to bacterial contamination, a main cause of food-borne diseases, also results in US\$65 billion in product losses annually to retailers and consumers. Detecting such bacteria is difficult, especially for the consumer. A package of ground beef may be acceptable at a supermarket, but the bacteria may reproduce rapidly after it is purchased.

[0010] Though food processors test for bacteria – some people complain that the testing is inadequate – food may leave a processor with undetectable bacteria levels. The bacteria reproduce rapidly if the food is transported at a warm temperature, kept on a warm loading dock at a market or restaurant, kept in a consumer's trunk while driving home,

maintained too long in a refrigerator or allowed to warm to room temperature before cooking. Unfortunately, meat contaminated with *E. coli* usually looks and smells normal. The United States government warns consumers and restaurants to cook ground beef to 160°F (71°C), the temperature that kills the *E. coli*.

[0011] Most food safety experts concentrate on the following nine principal bacteria:

[0012] *Campylobacter jejuni* is found in intestinal tracts of animals and birds, raw milk, untreated water and sewage sludge. Most transmission is from contaminated water, raw milk, and raw or under-cooked meat, poultry or shellfish. *Campylobacter jejuni* is a gram-negative bacterium like bacilli. It uses oxygen as its final electron acceptor.

[0013] *Clostridium botulinum* is widely distributed in nature including soil and water, on plants and in intestinal tracts of animals and fish. It grows only in little or no oxygen. Transmission usually is from improperly canned foods, garlic in oil and vacuum-packaged and tightly wrapped food. Infection can be fatal in three to ten days if not treated. *Clostridium botulinum* is a gram-positive bacterium typically rod-shaped and arranged as singles, pairs, or chains. The spores grow under favorable conditions (anaerobiosis and substrate-rich environments) and produce toxins as they rapidly propagate.

[0014] *Clostridium perfringens* is found in soil, dust, sewage and animal and human intestinal tracts. It grows only in little or no oxygen. It is called “the cafeteria germ” because many outbreaks result from food left for long periods in steam tables or at room temperature. Cooking destroys the bacteria, but some toxin-producing spores may survive. *Clostridium perfringens* are non-motile, rod-shaped, gram-positive bacteria. It is an anaerobic bacterium, which acquires energy by performing anaerobic respiration using nitrate as its electron acceptor.

[0015] *Escherichia coli* O157:H7 is found in the intestinal tracts of some mammals, raw milk and unchlorinated water. It is one of several *E. coli* strains causing human illness. It usually is transmitted through contaminated water, raw milk, raw or rare ground beef, unpasteurized apple juice or cider and uncooked fruits and vegetables. Person-to-person transmission also can occur. *E. coli* serotype O157:H7 is a mesophilic, gram-negative rod-shaped (bacilli) bacterium. It can perform complicated metabolism to maintain its cell growth and cell division and possesses operons for transport and utilization of sucrose, urease, and sorbose.

[0016] *Salmonella* (over 1,600 types) can be found in the intestinal tract and feces of animals and in raw eggs. *Salmonella* transmission comes from raw or undercooked eggs, poultry, seafood and meat and from raw milk and dairy products. *Salmonella Enteritidis* is a rod-shaped, gram-negative, proteobacteria that is non-motile. These facultative anaerobes are well adapted to survive in conditions with or without oxygen, allowing them to live in diverse environments. *S. Enteritidis* require glucose to survive and uses mixed acid heterofermentation of glucose to produce energy. This method of metabolism releases carbon dioxide and hydrogen gas as bi-products.

[0017] *Streptococcus Group A* is found in noses, throats, pus, sputum, blood and stools of humans. It is transmitted from people to food from poor hygiene, ill food handlers or improper food handling. Many outbreaks come from raw milk, ice cream, eggs, lobster, salads, custard and pudding allowed to stand at room temperature for several hours between preparation and eating. *Streptococcus Group A* is a gram-positive bacterium, which is nonmotile and does not form spores. Rather than using aerobic or anaerobic respiration, Group A *Streptococcus* uses fermentation, a metabolic pathway of lesser efficiency, which makes the cells grow more slowly.

[0018] *Listeria monocytogenes* exist in intestinal tracts of humans and animals, milk, soil, leaf vegetables and processed foods. It can grow slowly at refrigerator temperatures. It is transmitted from soft cheese, raw milk, improperly processed ice cream, raw leafy vegetables, meat and poultry. It causes illness itself instead of from toxins. *Listeria monocytogenes* are gram-positive rod-shaped bacteria that form single short chains. They do not form spores or branch and are motile via peritrichous flagella at room temperature (20°C – 25°C).

[0019] *Shigella (over 30 Types)* is found in human intestinal tract. They are rare in other animals. It is transmitted from person to person by a fecal-oral route and by fecal contamination of food and water. Most outbreaks result from food, especially salads, prepared and handled by workers using poor personal hygiene. *Shigella* is a non-spore-forming, gram-negative bacterium that aids in the facilitation of intracellular pathogens. *Shigella* pathogens use a mixed acid fermentation pathway to metabolize substrates. Products of this anaerobic pathway include ethanol, acetic acid, lactic acid, succinic acid, formic acid, and carbon dioxide.

[0020] *Staphylococcus aureus* is found on humans (skin, infected cuts, pimples, noses, and throats). Most transmission occurs through improper food handling. It multiplies rapidly at room temperature to produce a toxin that causes illness. *Staphylococci* are spherical gram-positive bacteria, which are immobile and form grape-like clusters. The central routes of glucose metabolism are the Embden-Meyerhof-Parnas (EMP) pathway and the pentose phosphate cycle. Lactate is the end product of anaerobic glucose metabolism, and acetate and CO₂ are the products of aerobic growth conditions.

[0021] Testing for molds is usually very different. Most molds grow in damp, dark regions of buildings or on food. Molds can adversely affect the buildings, e.g., by eating through walls, floors, ceilings and internal supports. Molds also produce spores that may be toxic to people. Pulmonary hemorrhage/hemosiderosis, and aspergillosis are examples of diseases associated with mold exposure.

[0022] Molds require spores to reproduce and spread. Ambient indoor and outdoor air contains large quantities of mold spores. When they land on damp soil or vegetation outdoors, they grow. However, the applicants' device and process are concerned primarily with mold growing on damp indoor surfaces and on food, or spores transported through building air ducts. Bread, especially artisan breads made without preservatives, is attacked by molds such as *Neurospora crassa*. Usually, mold growth is visible, and the offending food discarded. Other food mold is not as easily detected. *Aspergillus flavus* is a common mold on peanuts and may be present on other legumes and grains. That mold generates the toxin aflatoxin, which is a carcinogenic and which can be deadly if enough is eaten.

[0023] Though most food mold is easily detected by looking at the food, a mold growing in buildings may be hidden behind walls, in attics, in ducting and elsewhere. Eliminating all mold and spores in buildings is nearly impossible. Most preventive measures concentrate on eliminating excessive moisture or accumulated water, but a building owner may easily overlook a dripping pipe, water condensing from a cool surface or even a child's water spill on the carpet.

[0024] Mold detection usually relies on sight and smell. Many molds smell bad, so people often determine molds' presence by smell. Mold colonies may be visible unless they are behind walls, above ceilings, under flooring or in other inaccessible locations. Occa-

sionally, people resort to monitoring building air especially if the occupants are sensitive to small quantities of building mold.

[0025] Air monitoring requires sampling of indoor and outdoor air simultaneously. Because mold spores are everywhere, a high concentration of indoor spores becomes relevant only if the concurrent outdoor sample concentration is lower. However, most experts believe that routine air sampling is not beneficial because visual and scent mold detection usually works. But relying on scent to locate a mold may be difficult. In addition, many molds are difficult to detect through standard tests. Therefore, air sampling can yield false negative results.

[0026] Identifying the particular mold usually is not crucial because remediation techniques are similar for most molds. However, identifying the particular bacteria infecting a person or animal usually is important because particular antibiotics target particular bacteria.

[0027] Instead of relying on Petri dishes for microorganism detection, one can consider using various electronic devices for microbial detection including:

Frey, U.S. Publ. Appl. No. 2004/0014054

Ikeda, U.S. Pat. No. 5,565,085

Stetter, U.S. Publ. Appl. No. 2002/019265.

Thewes, U.S. Publ. Appl. No. 2004/0045839

Yang, U.S. Publ. Appl. No. 2004/0050694

Gelbart, U.S. Publ. Appl. No. 2004/0038420

Huang, U.S. Pat. No. 6,738,734

Andersson, U.S. Publ. Appl. No. 2004/0002167

Glasel, U.S. Publ. Appl. No. 2004/0106162

Bodenhamer, U.S. Publ. Appl. No. 2002/0045200

[0028] Conventional biological or microbial fuel cells convert biochemical energy directly into electricity. The driving force of a biological fuel cell is the redox reaction of a carbohydrate substrate such as glucose and methanol using a microorganism or an enzyme as the catalyst. Standard fuel cells rely on noble metals as their catalysts, but the catalysts in biological fuel cells are microorganisms or enzymes. Working conditions in biological fuel cells are mild, i.e., neutral solutions at or near room temperature.

[0029] Microbial fuel cells have several, potential uses. Using microbial fuel cells to generate electricity for power is one of the more talked-about applications. Much wastewater contains organic material, which can drive microbial fuel cells. The bacteria would serve two functions, consuming waste for purification and generating electricity. Microbial fuel cells also can act as a sensor to test wastewater for organic materials. Not all bacteria generate the same electrical output in microbial fuel cells. Therefore, microbial fuel cells for power generation typically concentrate on a limited type of bacteria. The fuel cells also are costly, rigid, and semi-permanent. Rather than generating electricity for power, applicants rely on microbes producing measurable signals, or characteristic signals indicating known pathogens under controlled conditions such as temperature and the type of carbohydrate food source.

SUMMARY

[0030] Applicants' sensor ("biosensor") uses the metabolic reactions of bacteria or other microbes to produce a quantifiable and measurable voltage signal. An electronic processor, recorder or other device reads and records such signals. The biological sensor is inexpensive and disposable and is useful for detecting living pathogens or other living substances. The sensor or detector can be made small ($< 1 \text{ mm}^2$ or smaller) to large ($> \text{multiple m}^2$) and can be constructed from inexpensive and abundant materials. Information from the device could lead to determining the presence or the particular type and kind of pathogen(s) present.

[0031] The operational principals of the biosensor senses electrons transferred during metabolic biochemical reactions resulting from microbial and bacterial metabolism. It is known and well understood that bacteria metabolism involves electron transfers, and bacteria act as the catalyst during metabolic chemical breakdown of carbohydrates. These microbial fuel cells require a positive (cathode) and negative (anode) electrode inside the device, and they can be constructed in a dual- or single-chamber configuration.

[0032] The dual-chamber configuration consists of two half-cell reactors separated by a polymeric proton exchange membrane (sometimes called "cation exchange membrane"). The proton exchange membrane should be hydrated with H_2O to function properly. The membrane conducts protons from the anode to the cathode but insulates passage of O_2 gases and electrons.

[0033] The anode chamber contains the negative electrode, and the cathode chamber contains the positive electrode. Oxidation occurs in the anode chamber (electron loss), and reduction occurs in the cathode chamber (electron gain). The proton exchange membrane separates the two chambers and allows protons to migrate freely by electrostatic forces from the anode chamber to the cathode chamber. The proton exchange membrane also prevents oxygen from the cathode chamber to migrate back through the proton exchange membrane to the anode chamber. Proton exchange membranes typically are made of Nafion with pore sizes about of 1.5 nm ($0.015 \text{ }\mu\text{m}$) and made of sulfonated tetrafluoroethylene-based fluoropolymer-copolymer. Other types of membranes may be acceptable. Proton exchange membranes made of Tetratex (ePTFE), also are

cost effective and durable. They have pore hole sizes from 0.05 μm to 7 μm in size. One company, Spectrum Labs, publishes a Relative Size Chart comparing pore hole sizes and typical common microscopic items. See

<http://spectrumlabs.com/filtration/PoreSize.html> (accessed Jan. 23, 2019).

[0034] The anode and cathode chambers operate in aqueous conditions, but the anode chamber is maintained in a low- or no-oxygen environment. Bacteria metabolizing inside the anode chamber operate in “anaerobic” conditions (i.e., without oxygen). Many bacteria thrive anaerobically including: obligate anaerobe bacteria, facultative anaerobe bacteria, microaerophilic anaerobe bacteria and aerotolerant anaerobe bacteria.

[0035] During metabolism, the bacteria can transfer electrons to the anode by chemical mediators, direct membrane associated electron transfer, or by “nanowire” linkages extending from the bacteria cell wall to the anode.

[0036] The biosensors may have two “half-cell” reactions that are catalyzed by the metabolic presence of bacteria or other microbes within the sensor. A single-chamber alternative also is contemplated. Inside the sensor, a substrate or carbohydrate material is added that is advantageous to the growth of bacteria or other microbes. The device can use specific carbohydrates if a physician suspects specific microbes.

[0037] Applications are numerous including pathogen detection in medicine and surgery, food and food packaging of meats, fish, dairy and other foods, residential and commercial buildings for mold detection or other pathogens (including airborne). Law enforcement, the military, homeland security and emergency responders also have applications. The biosensors have other hospital uses besides direct use with patients. Hospitals are concerned with detecting airborne diseases from air conditioner vents. Hospitals maintain detailed nosocomial rate indexes to measure the rate of infections (from unknown sources) present in their hospital.

[0038] Each bacterium has its own specific growth rate under controlled fuel-cell conditions and temperatures. Therefore, each produces its own specific voltage versus time growth-plot. To differentiate between different bacteria, biosensors in an array of sensors may have different substrates and different inhibitors. Inhibitors are weak, specific antibiotics that prevent growth of specific bacteria. A semipermeable membrane (called the proton exchange membrane) inside the biosensor, which can transfer hydrogen ions

(protons) given off by the metabolizing microorganism, separates the sides of the biosensor. By knowing the voltage versus growth-time versus temperature for the more common bacteria, a system including the sensors may use algorithms to analyze outputs from an array of micro-sensors. The results can narrow unknown bacteria down to one or more probable candidates.

[0039] One can use the same or similar techniques to detect molds or other living substances. Although much of this patent applications uses the term “microbe” and deals with bacteria, the discussions deal with molds too. To detect the growth of molds, the sensor uses substrate materials that are advantageous to mold. In the mold sensor, antibiotics may be added to the substrate to decrease the chance or eliminate false-positive detections of bacteria. Initial building construction or later construction can install these arrays at predetermined locations in homes or commercial buildings. For example, the arrays could be attached at the baseboard of the walls or on floors below carpet padding to detect if mold occurs due to accidental flooding. In these examples, the sensors remain dormant until they are activated by mold growth.

[0040] Determining sufficient mold spores without determining the species of mold might be sufficient. However, to determine a specific mold, the present device may use particular substrates and inhibitors to determine which molds are present. By similar methods used for bacteria detection and determination, the device may use an array of sensors. Each sensor is designed for a particular mold species. By analyzing the signals from each sensor, the system determines which mold is present by a process of elimination.

[0041] For medical and hospital use, bandages could include an array of disposable sensors. Sensors may be embedded into a patient during or after surgical procedures. These embedded sensors may be small (needle size), which may facilitate their removal after healing or after the critical period of concern. Though applicants discuss physicians and human patients, veterinarians can use similar sensors on animals.

[0042] Other applications of the biosensor device and system include inexpensive and disposable sensors for the food industry to determine whether meats, cheeses, other dairy products and other food are spoiling. Integrating a small, inexpensive sensor into food packaging could detect bacteria and register a tiny voltage. Applying the voltage to voltage sensitive paper could change the paper's color (e.g., green to yellow to red).

Green might mean that no bacteria are detected; yellow could mean that some detection of bacteria is present, and red could signal that the food is spoiled. Such a sensor could save food from early disposal after the “use-by date” when the product remains fresh. The absence of bacteria may not be the only reason to reject food after a date. Conversely, products exposed to lack of refrigeration and then re-refrigerated may spoil even before their printed expiration date.

[0043] Cells of organisms undergo metabolic processes yielding a transfer of electrical charges to and from their membranes to the environment. Therefore, they produce electrical energy during metabolism and reproduction. When these cells are incubated in specialized devices or specialized biochemical fuel cells, the charges generated over time produce a detectable voltage and current. The electrical output from these devices is typically micro-amps with millivolt potentials. Further, an increase in electrical signal output indicates a growth of organisms. Individual or small numbers of pathogens are often harmless because the body can destroy them. However, the uncontrolled replication by mitotic reproduction is often dangerous to the host. Bacteria and other pathogens typically divide mitotically every 15 minutes to 2 hours, increasing their numbers geometrically and multiple times before their death. The accumulated and summed electrical output is measurable and increases in relatively short periods.

[0044] By categorizing the electrical output or “growth” signals for various living substances along with various pathogens in unison and in combination, the background signal from normal metabolic processes can be subtracted from the total signal potential when unwanted pathogens are present and multiplying. Further, by categorizing the magnitude of this electrical growth potential for various pathogens in unison and together, the resulting growth potential can predict quantitatively the presence of the particular pathogen(s) present because the growth increase is different and can be known for different pathogens at different temperatures.

[0045] It may be important that the categorized growth rate is also determined relative to the surrounding temperature because temperature affects the pathogen growth rate. Knowledge that only certain pathogens exist in certain environments and temperatures aids the method. The categorical knowledge from the measured output signals strongly indicates the type or kind of pathogen present by a process of elimination when measur-

ing the growth rate signal at measured temperatures and comparing these signals to the baseline categorical database signals. In this way, it is possible to predict with higher probability the specific pathogen(s) present by subtracting out the baseline signal at the measured temperature and comparing it to the pathogen databases.

[0046] The technique for measuring the electrical signal output from the unknown pathogen or microorganism involves strategically placing the sensor(s) in the area of interest and waiting for the random migratory passage of the pathogens from their present location into the sensor(s). Because of this uncertainty when, or whether, a pathogen will encounter the sensor, choosing the size, placement and number of sensors should increase the probability of detecting any pathogens. Making the sensors of varying sizes also may increase the probability of detection. Therefore, sensors may range in size. Some may cover larger surface areas. This flexibility assists in enhancing the probability of measuring the presence of a pathogen.

[0047] Applicants contemplate that sensors could be made as small as the diameter of a surgical needle using nanotechnology. Therefore, one or more may be implanted into human or other living tissue as “real-time” incubators with the ability to measure and or detect the growth of bacteria. Similarly, miniaturizing the sensors could allow an array of sensors to remain small and manageable.

[0048] Each bacterium has its own specific growth rate under controlled fuel-cell conditions. Therefore, each produces its own specific voltage versus time growth-plot. Sensors for each microbe could have a different substrate that is advantageous to a specific bacterium. Specific antibiotics act like specific inhibitors to the individual bacteria. Therefore, an array of sensors with different substrates and different inhibitors could differentiate among different bacteria. By knowing the voltage versus growth-time for the most common bacteria, the process can use an algorithm that reviews the outputs from a plurality of sensors and eliminates microbes from sensors that generate no signals or signals below a threshold. Therefore, the method narrows the unknown microbe down to the most probable candidates.

[0049] One proposed sensor involves the utilization of two separate cavities – one containing an anode (negative terminal) along with the pathogen and a food source (such as carbohydrates), and one containing a cathode (positive terminal) with moisture (wa-

ter) – separated by a semipermeable membrane (proton exchange membrane) allowing charge transfer across the semipermeable membrane. A single chamber device also is contemplated.

[0050] The sensors can use inexpensive, disposable and flexible materials. The biosensors may be made by laminating flexible, thin, porous plastic or other sheets together with internal electrodes, anode and cathode, separated by a semipermeable membrane (proton exchange membrane) between the electrodes. However, unlike conventional biochemical fuel cells, the outer, thin sheets surrounding the anode use porous materials to allow pathogens to migrate into the anode to act as the catalyst if pathogens are present to sense the electrical signal occurring as the pathogens metabolize the carbohydrates or glucose fuels seeded in the anode cavity. Similarly, porous materials surrounding the cathode allow the free exchange of gases and oxygen into the cathode chamber.

[0051] The porous material could be Gore-Tex[®], an expanded and porous polytetrafluoroethylene (ePTFE), or a similar material. Typically, Gore-Tex used for clothing has about 9 billion pores/in² or around 1.4 billion pores/cm². Thus, the average pore has a length of about 11 μ-in or 27 μ-cm (0.27 μ-m). Typical smaller bacteria have dimensions of about 8 μ-in or 20 μ-cm (0.20 μ-m), semipermeable membranes made with ePTFE having pore sizes used in clothing would probably form biofilms and block most all bacteria entrance into the biosensor. See Trobos M, Juhlin A, Shah FA, Hoffman M, Sahlin H, Dahlin C. In vitro evaluation of barrier function against oral bacteria of dense and expanded polytetrafluoroethylene (PTFE) membranes for guided bone generation. Clin Implant Dent Relat Res 20:738-748 (2018). It also is necessary to have moisture (water) enter the biosensor through the semipermeable membrane (hydrophilic); therefore, typical ePTFE used for clothing would not work. However, PTFE used as alloplastic implants showed bacteria (e.g., Streptococcus aureus, Escherichia coli, etc.) can penetrate through the membrane or inhabit the microporous network of ePTFE. It has been reported that bacteria with a size of 0.5-1.5 μ-m can easily infiltrate the pores of ePTFE, which are stretched to 10-30 μ-m in size. See Jin YJ, Kang S, Park P, Choi D, Kim DW, Jung D, Koh J, Jeon J, Lee M, Ham J, Seo JH, Jin HR, Lee Y., "Anti-inflammatory and antibacterial effects of covalently attached biomembrane-mimic polymer grafts on Gore-

Tex implants.” *ACS Appl Mater Interfaces* 9:19161-19175 (2017). Therefore, ePTFE with large pores could be a suitable option as the outside semipermeable membrane. The top sheet 130 could also be made of a similar porous semipermeable material to allow O₂ exchange from the cathode end.

[0052] A signal processor such as a computer or other electronic device can read, analyze and process signals generated by the sensors. Instead of electronic analysis, the output of the sensor can act on disposable electro-photo-sensitive paper, which can change color based on accumulated charge. By “color,” applicants also mean shades of color or gray. The color sensor could be built into the top portion of the sensor itself. With this embodiment, the sensor could be useful for medical bandages or packaging for perishable foods. Using color sensors sensitive to different voltages or signals, one might use an array of voltage sensitive papers sensitive to different voltages to determine which microbe is present.

[0053] The system also could amplify the output signals into a recording device for data logging and post-processing. This approach could be useful for real-time data collection in Petri-dish laboratory growth samples or recording of data from air conditioner vents in buildings, hospitals or even residences.

[0054] The sensors may be connected electrically parallel or in series. There may be benefit to series connections for amplifying the output signal by summing the signal from multiple sensors.

BRIEF DESCRIPTION OF THE DRAWINGS

[0055] Fig. 1 is a schematic of a conventional biological fuel cell.

[0056] Fig. 2 is a side, sectional view of applicants' two-chamber biological fuel cell sensor.

[0057] Fig. 3 is a plan view showing an array of applicants' biological fuel cells.

[0058] Fig. 4 is a schematic of any array of applicants' biological fuel cells in electrical series.

[0059] Fig. 5 is a chart showing the size range for various microbes, other molecules and atoms.

[0060] Fig. 6A is a perspective view of an adhesive bandage with applicants' biological fuel cells. Figs. 6B and 6C shows the bandage applied to the back of a person's hand. In Fig. 6C, the change in color indicates detected microbes.

[0061] Figs. 7A and 7B are figures of a food package containing meat and applicant's biological fuel cell being exposed to potential contamination from microbes. Fig. 7B shows the food being removed from the package, and a portable device can read the output of the fuel cell.

[0062] Fig. 8 is a schematic of a one-chamber biological fuel cell.

[0063] Figs. 9 and 10 also are schematics of a one-chamber biological fuel cell

[0064] When detailed descriptions reference one or more drawing figures, the element being discussed is visible in that drawing. The element also may be visible in other figures. In addition, to avoid crowding of reference numerals, one drawing may not use a particular reference numeral where the same element is in another drawing with the reference numeral.

DETAILED DESCRIPTION

[0065] A microbial fuel cell MFC is a biological reactor that converts chemical energy present in the bonds of organic compounds into electric energy through the catalytic reactions of microorganism, typically in anaerobic conditions. They use biocatalysts for the conversion of chemical energy to electrical energy.

[0066] Microbial fuel cells share similarities with conventional fuel cells, but instead of relying on inorganic catalysts like platinum or other noble metals, they use biocatalysts such as enzymes or whole living organisms as catalysts for converting chemical energy into electricity.

[0067] Microbial fuel cells can operate in two ways. They can use biological catalysts – enzymes extracted from biological systems – to oxidize fuel molecules at the anode and to enhance oxygen reduction at the cathode of the fuel cell. Whole microbial cells also can be catalysts in the fuel cells. In both cases, electrical coupling of the biological components of the system with the fuel cell's electrodes must occur. Molecules known as electron-transfer mediators can provide efficient transport of electrons between the biological components, enzymes or microbial cells, and the anode electrodes of the fuel cell. Further, integrated biocatalytic systems that include biocatalysts, electron-transfer mediators and electrodes have been recently developed and utilized in fuel cells. The typical chemical reactions inside the anode and cathode chambers for anaerobic bacteria include $C_xH_yO_z + H_2O \rightarrow CO_2 + e^- + H^+$ (anode) and $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ (cathode).

[0068] The Fig. 1 schematic shows two chambers, cathode chamber 12 and anode chamber 14. The cathode chamber contains cathode 18 in a liquid medium such as water or hydrogen peroxide. Likewise, the anode chamber contains anode 20 suspended in a liquid medium. Bacterium 16 is suspended in the anode chamber's medium. A conductor 22 electrically connects the anode and cathode through a load (resistor). Electronics or other devices (not shown) can read the current or voltage.

[0069] Nicotinamide adenine dinucleotide (NAD) is an important cell coenzyme. In a biological redox (oxidation-reduction) reaction, NAD interconverts between reduced nicotinamide adenine dinucleotide (NADH) and oxidized (NAD⁺) forms. The removal of hy-

drogen atoms accomplishes most oxidation. For NAD, the reaction is $\text{NAD}^+ + 2\text{H} \rightarrow \text{NADH} + \text{H}^+$. Fig. 1 shows the reaction schematically including the H^+ ions 24.

[0070] Electron-transfer mediators MED may also be present to assist the reaction. Some microbes are more resistant than others are to the redox reaction and may need mediators to help strip off electrons.

[0071] NAD also is involved with glycolysis. Glucose oxidizes to pyruvic acid. NAD^+ is the oxidizing agent, which reduces to NADH. Pyruvate oxidizes further to CO_2 , 26 in Fig. 1.

[0072] This is a simplified explanation. Some of it applies only to aerobic metabolism, and the redox reaction may work differently for anaerobic microbes. But the reaction generates ions, which creates an electrical potential between the anode and cathode, which generates current through conductor 22.

[0073] The redox reaction varies in different microbes. Though the redox mechanism is similar, the rate of the reactions varies and depends on the cell's metabolism. Therefore, the voltage between the anode and cathode varies depending upon microbe type, temperature and growth time.

[0074] The dual-chamber sensor works by forming two half-cell chambers separated by a semipermeable membrane (proton exchange membrane). The reaction can occur in applicants' sensor such as sensor 100 (Fig. 2). This sensor has two, half-cell chambers, anode chamber 110 and cathode chamber 130. The anode chamber has an outer semipermeable wall 112 that may be formed of flexible material, allowing free passage of microbes and moisture into the anode chamber. Likewise, the cathode chamber includes a semipermeable outer wall 132, allowing passage of oxygen into the cathode chamber. Semipermeable membrane 150 (proton exchange membrane) separates the anode and cathode chambers. The ends 114 and 116 of the anode chambers outer wall 112, attach to the respective ends 134 and 136 of the cathode chambers outer wall 132. By "attached," applicants mean that the ends attach directly to each other or that the ends of semipermeable membrane 150 may be intermediate at the ends. Those ends may be heat-sealed or otherwise attached to semipermeable membrane 150. As Fig. 2 shows, sensor 100 can be made thin, flexible, inexpensively and disposable, features that may be useful for many applications.

[0075] Anode 118 is in anode chamber 110. It may extend through outer wall 112 in Fig. 2. A wire or other electrical connector may extend through outer wall 112 and contact the anode. The anode is shown planar, but it can curve, especially near outer wall end 116. Similarly, cathode 138 in cathode chamber 130 may extend through outer wall 132 and may be attached to a wire or other electrical connector through outer wall 132 to contact the cathode. The cathode also can curve, especially near outer wall end 136. The anode and cathode connect to signal processor 140, which may be a computer, special electronics or another device for indicating current or voltage between the anode and cathode.

[0076] When choosing the type and kind of material for anode 118 and cathode 138, satisfactory attributes may include good conductivity, acceptable physical strength, high surface area, favorable surface properties, good chemical and electrochemical stability and low cost. Graphite fiber cloth, graphite fiber, felt and carbon fiber paper are candidates for the materials. A graphite powder could be sprayed or brushed onto a semipermeable membrane for the anode or cathode. Permanent anodes and cathodes used in biochemical fuel cells, which are designed for generating electricity are usually designed to be permanent. Therefore, their design must account for longer-term use. Applicants' anodes and cathodes are in disposable devices that may operate for a few days more or less. Cost will be important, but the anodes and cathodes cannot deteriorate or break before the sensor must be used. Having the outer membrane surfaces surrounding the anode and cathode electrode surfaces made of a semipermeable material is important to the use and operation of the biosensor because pathogens and moisture need access paths into the sensor on the anode side and oxygen and gas exchange on the cathode side.

[0077] In addition, current output is a function of the surface area of the anode and cathode. Because the sensors can be made small, and the output voltage is small, the anode and cathode have a relatively large surface area in the small space of the sensors' chambers 110 and 130.

[0078] At least a portion 120 of outer wall 120 of the anode chamber 110 is a porous semipermeable membrane having pores large enough for ingress and egress of moisture and those microbes of interest such as bacteria 122. Fig. 5 shows the sizes of vari-

ous microbes. Knowing the sizes is a useful guide for choosing the minimum semipermeable membrane pore sizes. Bacteria are prokaryotes because they contain no cell nucleus, or any other membrane-bound organelles.

[0079] The semipermeable membrane may be Gore-Tex® or similar material. Using woven fabric treated to be hydrophilic on the outer surface and a hydrophobic on the inside surface is another possibility, but may not be necessary provided the anode chamber is maintained moist by the source. The semipermeable membrane holds liquid within chamber 110. Anode chamber may contain fluid passed through the semipermeable membrane from the source, or it may contain fluid before use. Membrane 120 provides the free-flow of fluid and microbes into the anode chamber. Anode chamber 110 also may contain glucose or carbohydrate 124 to attract (chemotaxis) and feed the microbes' metabolism.

[0080] Part or all of outer wall 132 of cathode chamber 130 also could have a porous semipermeable membrane.

[0081] Cathode chamber also contains liquid, preferably water or hydrogen peroxide, H_2O_2 . Under certain configurations aerobes, organisms that require oxygen or that live in the presence of oxygen, produce hydrogen peroxide naturally as a by-product of metabolism. Aerobes have enzymes that can decompose low concentrations of H_2O_2 to water H_2O and oxygen O_2 .

[0082] Semipermeable membrane 150 is a proton exchange membrane. They usually are made from ionomers. They conduct protons but do not allow gases to permeate. DuPont's Nafion is a commonly used proton exchange membrane. Gelatinous agar with salt also acts as a proton exchange membrane. Its low cost may make it suitable for applicants' sensor.

[0083] As the microbe metabolizes and reproduces, H^+ ions are generated. That generates an electrical potential between anode 118 and cathode 138, which causes a current to flow from the anode to the cathode.

[0084] The current probably will be small, in the order of micro-amps. The potential likely will be in the millivolts, but sensitive instruments can read the electrical signal. Instead of using electronic instruments, it may be possible to sense the generated electricity with color-changing paper or other chemical sensitive to current or voltage.

[0085] Standard biochemical fuel cells require adding glucose or other carbohydrates along with microbes to continue the redox reaction. Applicants rely on microbes passing from outside the device through the semipermeable membrane 120 into the anode chamber 110 to metabolize carbohydrates previously placed on the anode. Also, in the disposable version of the proposed biochemical sensors, applicants are proposing using the sensors for a relatively short active period (on the order of days) and so no new fuel source (carbohydrate) must be loaded within the sensor.

[0086] The sensors can be small. That creates a potential issue. If one sensor or a small array of sensors is placed on a wound, a portion of a wound could be badly infected while the area under the sensor could be relatively free of microbes. Similarly, one area could be infected with one pathogen, but the region near the sensor could have a different pathogen. The different pathogens might have dissimilar reactions to antibiotics a physician might prescribe.

[0087] Therefore, mounting separate sensors in a tightly packed array that covers the entire region of interest could minimize that problem. Fig. 3 shows such an array 200 of sensors 210. The sensors can be held together with adhesive. In Fig. 3, the array is on tissue 214. The drawing shows 55 sensors in the array. Applicants expect that the array could have more or less sensors. The larger array could be cut into smaller arrays. Cutting could damage sensors along the cut, but enough sensors will remain to provide sufficient readings. The electronics could ignore any sensors along the periphery of the array. A large array could have spaces between some sensors so cuts of larger arrays into smaller ones would be along the spaces.

[0088] Cover 218 (Fig. 3) can connect electrically to each sensor, or the connection can be elsewhere. The electrical output can be a sum of all the signals generated by the sensors 210. The electronics also could provide a readout for each sensor or for a group of adjacent sensors. For example, the sensors could be numbered 1, 2, 3 ... for the first row along the top in Fig. 3. The next row could be 7, 8, 9 The sensors can be linked. For example, the signal from adjacent sensors 1, 2, 3, 7, 8 and 9 could be combined, and the signals from other groups of sensors also could be combined separately. Combining the output of six sensors is an example and may not be ideal. Using an array of adjacent sensor may help to locate an infected area.

[0089] Signals from non-adjacent sensors could be combined. If any group shows signals indicating infection, the physician would start an antibiotic regimen for the identified infection.

[0090] If the electronics monitor groups of more than one sensor, the sensors may be connected in parallel or in series. Connecting them in series may be preferable because the tiny voltages from each will add. Connecting the sensors in parallel yields more current, but a parallel connection probably is less desirable because the individual sensors can have mismatched voltages. However, the process used to manufacture the sensors should insure that no sensor is an open circuit. Even if a few sensors become open circuits, applicants believe that with sufficient sensors, the system can have enough redundancy to account for an occasional open circuit.

[0091] Fig. 4 shows a schematic of an array 300 of sensors in two rows. Sensors 310 are in one row, and sensors 312 are in the second row. In this arrangement, the voltage between the negative electrode 320 and the positive electrode is the sum of the voltages from all the sensors in array 300.

[0092] In Fig. 3, the sensors are round (shown as circular). An oblong shape may be desirable. Triangular, rectangular, hexagonal and other shapes can pack more tightly, but they have corners in which the reaction may not work as well. Therefore, circular or other rounded shapes may prove beneficial.

[0093] The two outer material sheets may be fabricated from a semi-porous material to allow the free egress of pathogens to pass into the anode chamber (where the carbohydrate food source is located) and allow fluids to pass freely through the device walls.

[0094] The sensor or array of sensors could be mounted in or on a bandage. Fig. 6A shows an array of sensors 410 mounted on an otherwise conventional adhesive bandage 400. The sensors in the array connect electrically, and the signal can be read at pads 412 and 414. The pads are shown on the inside of the bandage, but the bandage material would have openings (not shown) on the top side.

[0095] Fig. 6B shows such a bandage 400 on the back of a hand. Instead of using electronics to read a signal from the sensor array, applicants contemplate that electrical activity could change the color of the top surface 402 of the bandage. See Fig. 6C.

[0096] Fig. 7 shows the use of the sensor to monitor food spoilage using applicants' sensor. Meat, for example steak 500, is contained within a top and bottom sheet of material. Only a top sheet 510 is visible. A single sheet also may be satisfactory. The steak and sheet are within sealable container 530.

[0097] Scattered on the inside of the sheet(s) are sufficient numbers of applicants' sensors (not shown) to contact a sufficient portion the meat to detect spoilage. A steak is not homogeneous. It contains muscle meat, bone, fat and connective tissue. Ground meat is more homogeneous than steak, but a package may contain meat from different animals or from different parts of the same animal. Packaged chicken contains different animal parts such as skin. A package of fish may contain filets from different fish. Whole fish has a head, tail, fins, etc. Further, the size of the food may vary relative to the size of sheet 510. Therefore, the number of sensors may vary depending on the food. Conversely, the sheet could have enough sensors to be in contact with enough areas of the largest food.

[0098] Though the discussion focused on meat, poultry and fish, the system could extend to vegetables, dairy and other food. Sensors for dairy or other packaged food could be built into the container. They also could be attached to the packaging for fresh vegetables. Containers for home food storage could mount to the container.

[0099] As with previous uses for the sensors, the food could be monitored constantly. That is, the sensors could be electronically connected to electronic monitors. They also could use color change material. Continuous monitoring of hundreds of food packages in a supermarket likely is impractical. Instead of wired connections, the sensors could connect to an RFID chip that could transmit the sensor signals to a signal processor such as a computer or other local monitor.

[0100] RFID chips could also monitor mold sensors in regions of buildings by relaying measured signals from the mold sensors short distances back to a nearby staged control center. The control center may relay the aggregate room data back to a master control station for an entire building or group of buildings. If unknown water damage produces mold growth within a room or rooms of a home or commercial building, such an arrangement could pinpoint the infected location for quicker remediation and removal.

[0101] Another process uses a separate reader 520. Film 510 has two electrodes 512 and 514. When the sensors first contact the food, the sensors act like an uncharged battery because they lack microbes or pathogens within them that have passed into the anode chamber to “activate” the sensor and begin the metabolic action inside the anode chamber. When the microbial catalysts activate the sensor, the sensor takes time to “charge” to a measurable voltage. Reader 520 has two probes 522 and 524 that can contact the electrodes on the film. The reader may have on-off and function buttons 526. Fig 7B. Function buttons allow for selecting the food being monitored based upon the signal strengths from the bacteria that predominate on different foods. For example, E-Coli is a more common contaminate in meats, raw milk, uncooked fruits and vegetables, and Salmonella is less prevalent in vegetables but still found in meats and dairy products. Screen 528 on the reader has a display for showing what microbe is monitored. Metabolic activity also is displayed.

[0102] Reader 520 is a small electrical device that reads the electrical voltage output from the sensor films. The reader allows quick reads after microbes in the sensors have had time to incubate and generate an electrical potential. One could connect the reader temporarily connected to the output electrodes 512 and 514 to read any metabolic signal. The reader also could be clipped onto the packaging so electrodes 512 and 514 remain connected to the reader’s electrodes 522 and 524.

[0103] Although this arrangement is discussed for food monitoring, it also could be used for monitoring wounds, other medical applications and for mold detection.

[0104] Fig. 2 showed a dual chamber MFC. Fig. 8 shows the construction of a single chamber MFC 600. Anode 610 and cathode 630 may be made of conductive, semipermeable material. For example they may be carbon paper or carbon fiber cloth that also can be coated with graphite powder spray to facilitate the conduction of current. The anode and cathode should allow liquids to flow through them to permeate the MFC biosensor. The anode could also be coated with a carbohydrate substance as a fuel source and accelerator to attract the microbes (chemotaxis) to the anode if bacteria 612 are present. Proton exchange membrane 620 may be a coating or film of salt and agar. The spacing between the anode and cathode may vary. It is exaggerated in Fig. 8. There

may be a thin structural open-cell material (like foam) placed between the anode 610 and proton exchange membrane 620 to hold the two regions apart.

[0105] Metabolizing and reproducing microbes generate H^+ ions, which produces an electrical potential between anode 610 and cathode 630. The protons, H^+ ions, pass through the open-cell matrix and through the proton exchange membrane 620 to the cathode 630 and react with O_2 from the air to form H_2O . The reaction generates a potential between the anode and cathode. Current flows between the anode and cathode through load 650. The semipermeable membranes forming the anode and cathode allow water and air to pass from the outside into the MFC biosensor. Although not shown in Fig. 8, a tougher open-cell screen or permeable material may cover the outside of the anode and cathode to protect them. This tougher material also would be semipermeable to allow bacteria and water to reach the anode easily and air (oxygen) to enter the cathode, creating water on the cathode surface by the redox reaction with H^+ ions.

[0106] An alternative single chamber biosensor could have the anode and cathode made of semipermeable material coated with graphite powder spray to facilitate the conduction of current and moisture effusion into the biosensor from the source containing the unknown microbes. The anode could also be coated with a carbohydrate substance to facilitate a fuel source (chemotaxis) for microbe catalysts.

[0107] Good conductivity, acceptable physical strength, high surface area, favorable surface properties, good chemical and electrochemical stability and low cost are attributes to consider in choosing the type and kind of electrode material for the biosensor. Graphite fiber cloth, graphite fiber felt, carbon paper and graphite fiber brush are examples.

[0108] Figs. 9 and 10 are another way of looking at the single chamber MFC. In Fig. 9, a semipermeable cover 728 allows easy migration of microbes and water into the biosensor but also protects anode 726 on one side of chamber 720. Proton exchange membrane 722 is on the other side of the chamber, and cathode 724 is adjacent membrane 722. The proton exchange membrane may be Nafion. The anode may be formed of carbon paper, and the cathode may be carbon cloth. An opening 730 into chamber 720 might be provided to monitor the process.

[0109] In Fig. 10, chamber 700 is on a source 702 of bacteria such as meat, other food or a wound. Course, semipermeable membrane 712 is adapted to conform to source 702, and anode 710 mounts above the membrane and conforms to the membrane. Coarse foam separator 708 separates the anode from proton exchange membrane 706. Cathode 704 is above the proton exchange membrane.

[0110] The single chamber biosensor may be cost effective because it is thin, flexible, and inexpensive, and, therefore, disposable. The layers are flexible, laminar layers and not bulky rigid plastic like state of the art MFC fuel cells. The two electrodes 704 and 710 in Fig. 10 are typically made of carbon paper or carbon cloth connected to electrical wires or contact points 714 and 716 (thickness ranges: $\approx 0.004''$ to $\approx 0.1''$). Coatings may be applied to the electrodes to enhance electrical sensitivity. The anode is coated with a thin film of carbohydrate (glucose or other sugar) on the side touching the source.

[0111] A permeable membrane 712 covers the anode, but it may not be needed in all configurations (thickness ranges: $\approx 0.004''$ to $\approx 0.05''$ if used). The pore size of this permeable membrane is unimportant as long as it allows free migration of bacteria from the source to the sugarcoated anode and allows water (moisture) to transfer from the source to the anode and throughout the inside of the single chamber. The permeable membrane primarily provides physical protection of the anode. It could be made from similar screen material used to cover building windows, which may have square mesh sizes of about 0.05 inches. This mesh size easily passes bacteria and moisture into the single chamber.

[0112] Separator 708 between the anode and cathode prevents them from touching and electrically shorting. The separator could be a thin, coarse (open-cell) foam. Ideally, it is advantageous to make the separator thin to minimize the size of the single chamber biosensor while still electrically separating the two electrodes. Size ranges could be from $\approx 0.05''$ to $\approx 0.25''$ depending upon application and usage. If no proton exchange membrane 706 is used, the foam separator may need to be thicker.

[0113] Cathode 704 is exposed to air on one side and may have a thin proton exchange membrane 706 (typically $12\ \mu\text{m}$ to $250\ \mu\text{m}$ thick with pore holes about $0.015\ \mu\text{m}$ to $1\ \mu\text{m}$) attached to the opposite side of the cathode, i.e., inside the single chamber, towards the anode. The proton exchange membrane has a small pore hole size, which

allows protons to migrate across the boundary but prevents O₂ from migrating from the cathode to the anode.

[0114] If the source is a steak or other flat food, flexibility of the course membrane 712 and anode 710 may not be crucial because the biosensor can lie flat on the flat food forming a partial gas boundary between the biosensor and the source. This reduces the permeation of O₂ onto the anode and creates anaerobic conditions inside the anode chamber side. Flexibility is more important if the source is not flat to allow the membrane and anode to conform to the source's surface. If the sources are identically shaped, the biosensor can be designed to conform to the shape.

[0115] A thin sugar or carbohydrate coating may be added on the anode. Bacteria present on the source can migrate into the biosensor and attach to the anode by chemotactic mechanisms. ("Chemotaxis is the directional locomotion of cells towards a source of a chemical gradient."). When the bacteria reach the sugarcoated anode, the bacteria metabolize the sugars and activate or start the electron generation process of the MFC.

[0116] The semipermeable membrane surrounding the sugarcoated anode may not be necessary, other than for added physical protection of the anode. The pore size could be made much larger than the specified 10 μm to 30 μm reducing the impedance of the bacteria to migrate onto the anode.

[0117] The entire single chamber may be laminated on the edges or closed on the edges (as shown in the figure below) to hold the pieces together. Allowing the free migration of bacteria and fluids from the source to the anode and inside the single chamber is important. The anode region is placed on the source to minimize oxygen (air flow) between the source and anode-side of the single chamber. The opposite side of the single chamber (cathode side) is exposed to air for free-flow of oxygen.

[0118] The biosensor may be a one-shot device that stores the fuel (carbohydrate) inside and adjacent to the anode. It is not "loaded" with the catalyst (the microbes or pathogens). Instead, bacteria must be able to ingress into the biosensor to start the metabolic reactions. The outer membrane 712 must allow pathogens and some liquid to pass freely into the biosensor. Options for hydrophobic coatings on the inside surface of membrane 712 may be useful to prevent or limit fluids from passing back out. Otherwise, the fluids could contaminate food or wound.

[0119] The anode and cathode must conduct electricity, so graphite cloth is a possible membrane. Making the anode and cathode out of porous graphite paper or porous paper with graphite sprayed onto the surfaces may be a cost-effective alternative.

[0120] Any need for mediators to assist in transporting the electrons from the microbes during metabolism may be unnecessary because maximizing current output (power output) is not the principal goal. The biosensor only needs to generate enough power to register a signal indicating growing microbes' presence. This possible reduction or elimination of using mediators is helpful because many are costly, and some are toxic.

[0121] Typical anode chemical reactions occurring inside the anode and cathode chambers are: $C_xH_yO_z + H_2O \rightarrow CO_2 + e^- + H^+$ (anode) and $O_2 + 4H^+ + 4e^- = 2H_2O$ (cathode)

[0122] The benefits of the single chamber MFC biosensor include: a) reduced material, parts, and fabrication cost; b) higher cathode efficiency; c) higher power densities ($\approx 500 \text{ mW/m}^2$). Single chamber MFC's with air cathodes can operate with or without a proton exchange membrane. Depending upon separation distance between the anode and cathode, performance can be increased without a proton exchange membrane. The maximum power density and generated voltage increases without the proton exchange membrane because of reduced internal resistance. A single chamber biosensor also can be thinner, may produce a larger output signal for the same surface area sensor due to lower internal resistance, and may cost less.

[0123] The description is illustrative and not limiting and is for example only. Although this application shows and describes examples, those having ordinary skill in the art will find it apparent that changes, modifications or alterations may be made. Many examples involve specific combinations of method acts or system elements, but those acts and those elements may be combined in other ways to accomplish the same objectives. Regarding flowcharts, additional and fewer steps may be taken, and the steps as shown may be combined or further refined to achieve the methods described. Acts, elements and features discussed only in one embodiment are not intended to be excluded from a similar role in other embodiments.

[0124] "Plurality" means two or more. A "set" of items may include one or more of such items. The terms "comprising," "including," "carrying," "having," "containing," "involving," and the like in the written description or the claims are open-ended, i.e., each means,

"including but not limited to." Only the transitional phrases "consisting of" and "consisting essentially of" are closed or semi-closed transitional phrases regarding claims. The ordinal terms such as "first," "second," "third," etc., in the claims to modify a claim element do not by themselves connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed. Instead, they are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term). Alternatives such as "or" include any combination of the listed items.

WE CLAIM:

1. A single chamber microbial fuel cell comprising:
 - a. an anode adapted to be positioned near a potentially contaminated source to receive microbes on the source,
 - b. a cathode spaced from the anode, and
 - c. carbohydrate positioned between the anode and cathode.
2. The fuel cell of claim 1, wherein the anode is semipermeable.
3. The fuel cell of claim 1, further comprising an electrical connection between the anode and the cathode.
4. The fuel cell of claim 1, wherein the anode is flexible.
5. The fuel cell of claim 1, further comprising carbohydrate contacting the anode.
6. The fuel cell of claim 1, further comprising a separator between the anode and cathode.
7. The fuel cell of claim 6, wherein the separator is flexible and porous.
8. The fuel cell of claim 1, wherein the anode has an outer face adapted to face the source and further comprising a semipermeable membrane adjacent the outer face of the anode, the pore size of the semipermeable membrane being large enough to allow free passage of microbes and to allow fluids into the microbial fuel cell.
9. The fuel cell of claim 8 wherein the semipermeable membrane is a mesh screen.
10. The fuel cell of claim 1, wherein the cathode is exposed to air.
11. The fuel cell of claim 1, wherein the cathode is flexible.
12. A sensor for determining the presence of microbes on a potentially contaminated source comprising:
 - a. a microbial fuel cell having an anode adapted to be positioned near a potentially contaminated source to receive microbes on the source,

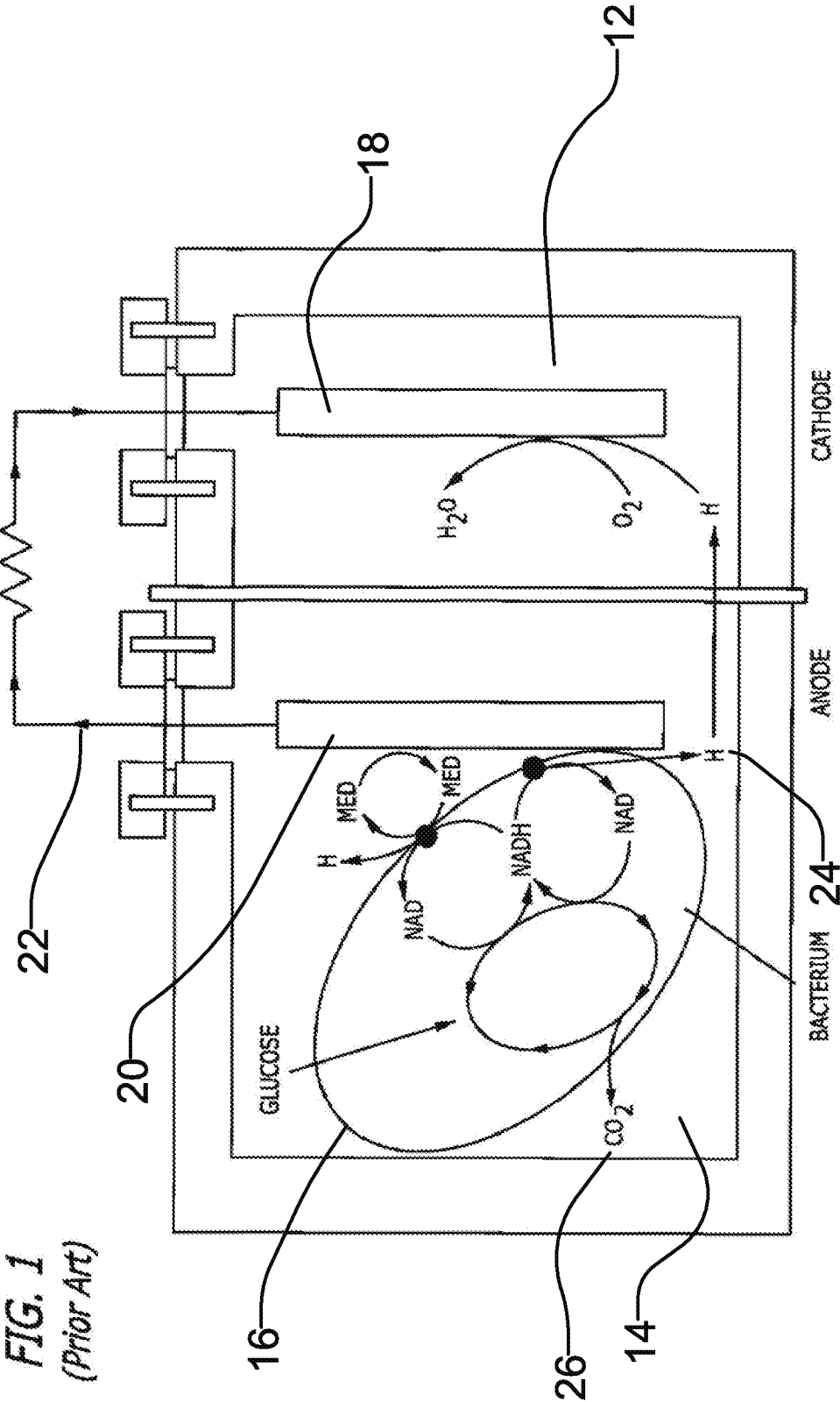
- b. a cathode spaced from the anode,
- c. carbohydrate positioned between the anode and cathode, carbohydrate is metabolized by microbes, generating an electrical signal.
- d. a signal processor capable of being electrically connected to the anode and the cathode, the signal processor reading and analyzing the signal.

13. The sensor of claim 12, wherein the signal processor comprises material that changes color when exposed to an electrical signal.

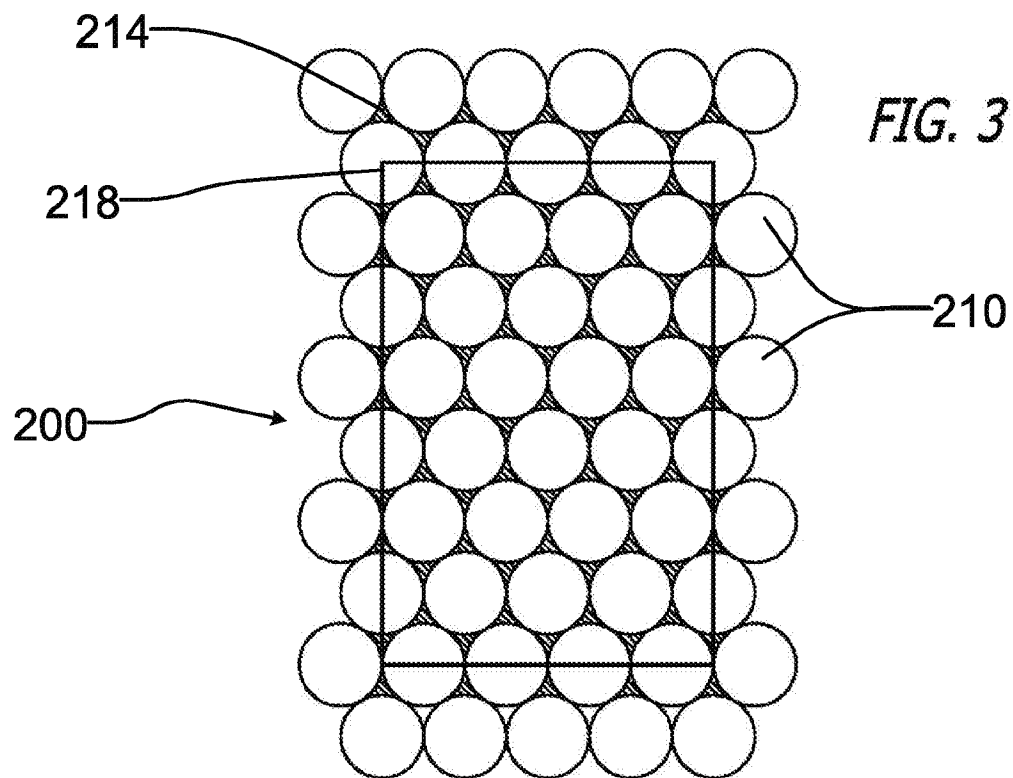
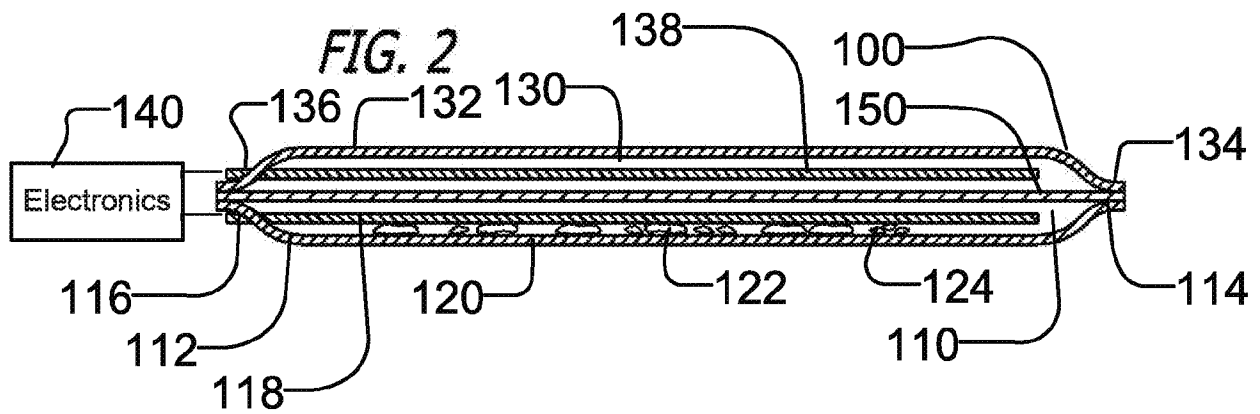
14. The sensor of claim 12, further comprising multiple fuel cells, an RFID device associated with each cell, wherein the signal processor is adapted to receive signals from the RFID devices.

15 A device for determining the presence of microbes on a potentially contaminated source comprising:

- a. a microbial fuel cell having an anode adapted to be positioned near a potentially contaminated source to receive microbes on the source,
- b. a semipermeable outer membrane between the anode and the source having constructed to allow microbes and moisture to freely pass into the sensor, the sensor generating an electrical signal when microbes pass into the sensor and metabolize,
- c. a cathode spaced from the anode, and
- d. carbohydrate positioned between the anode and cathode.
- e. a signal processor capable of being electrically connected to the anode and the cathode, the signal processor reading and analyzing the signal.



2/7



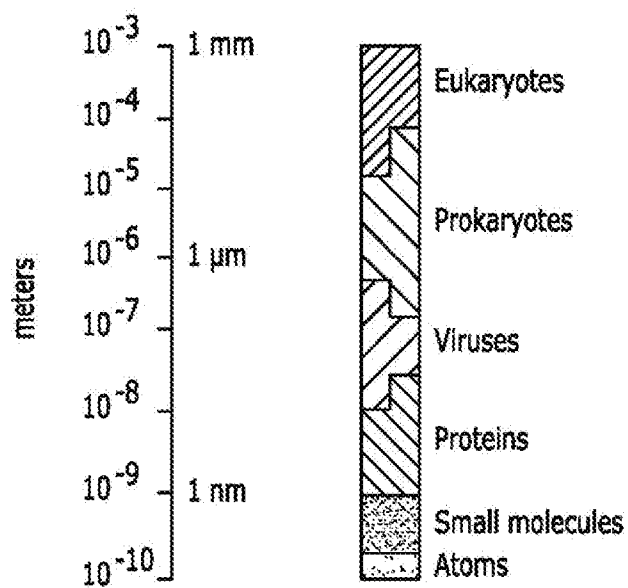
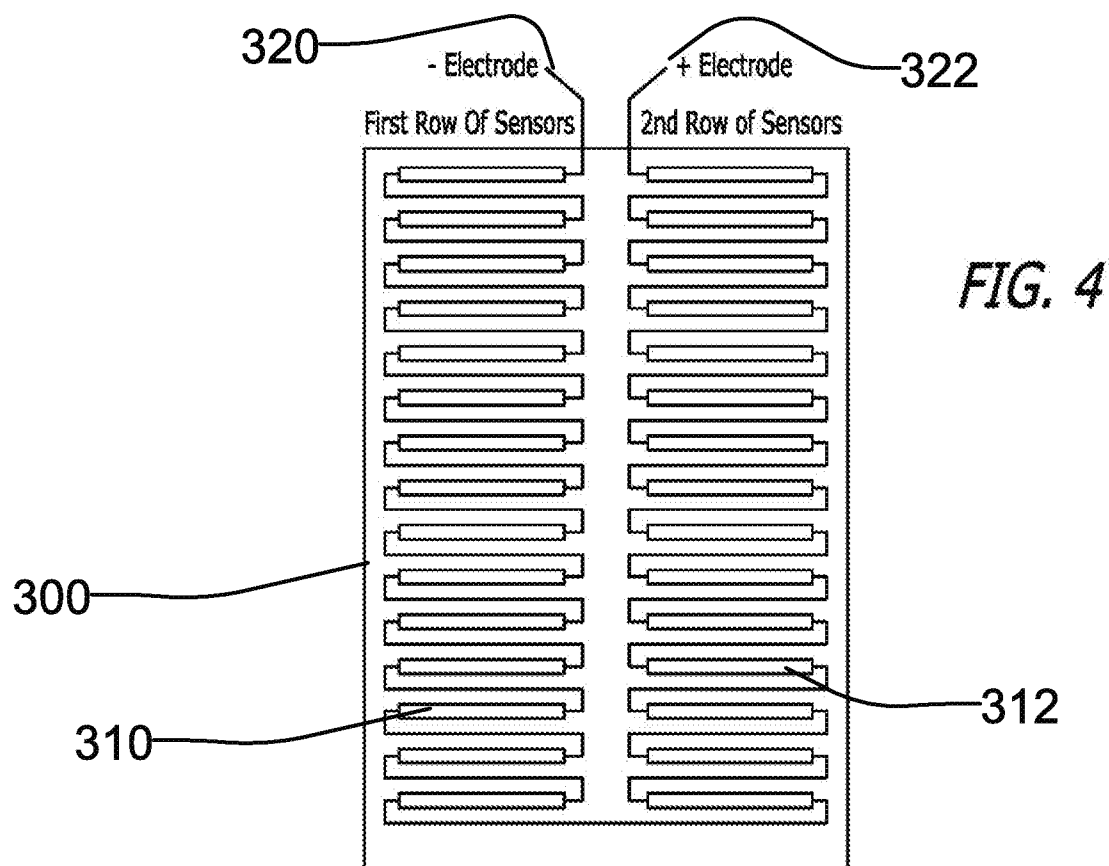


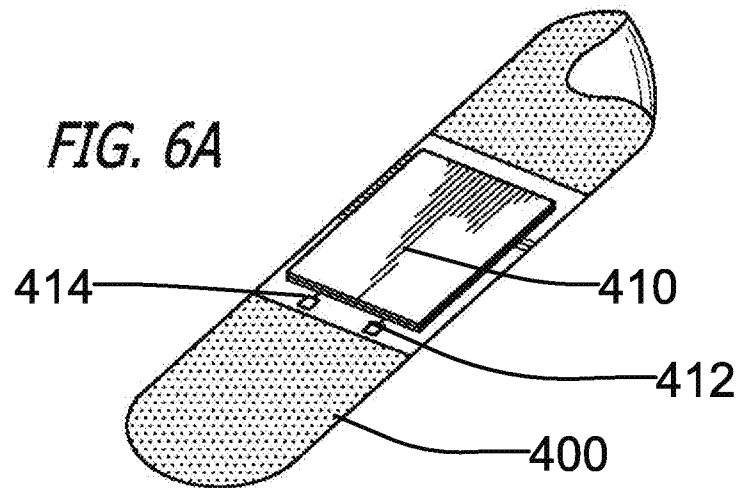
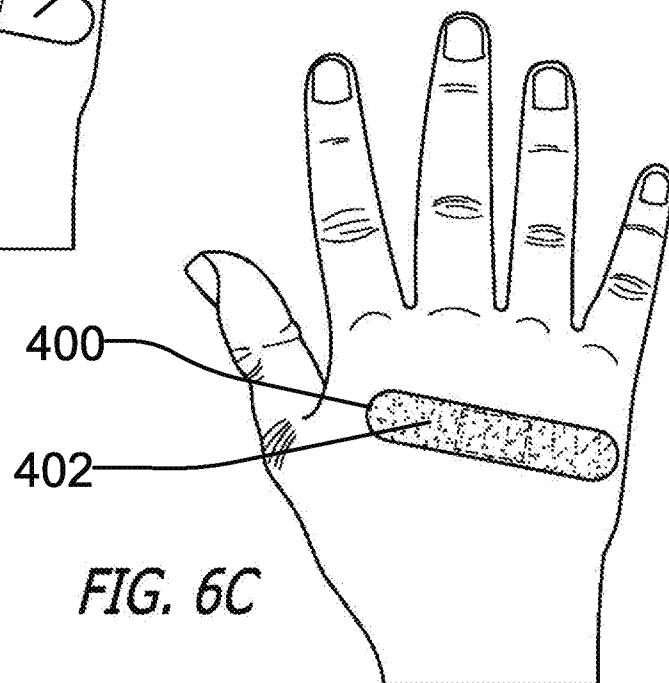
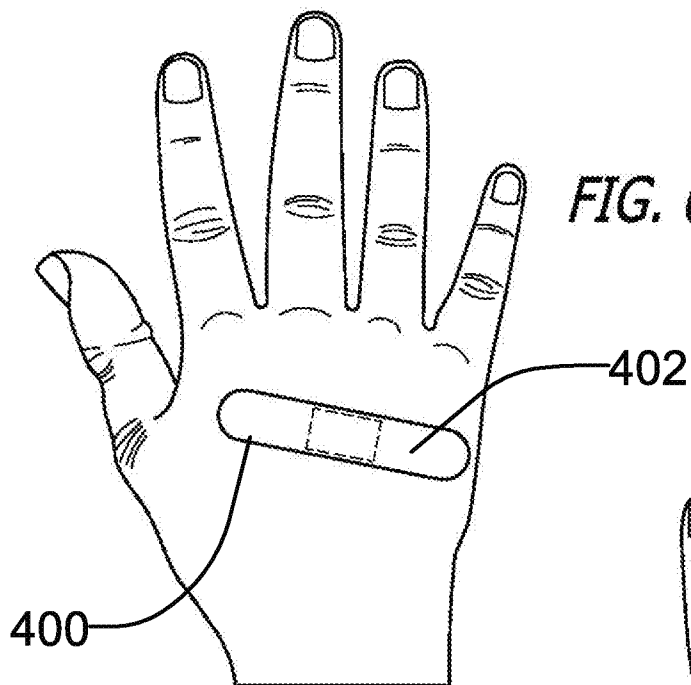
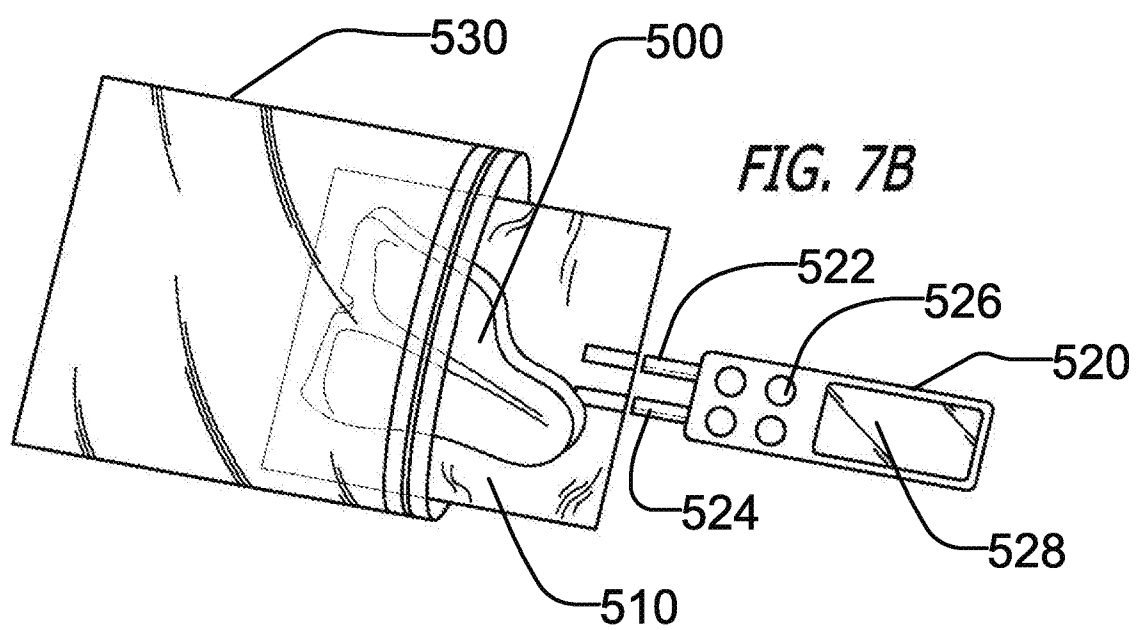
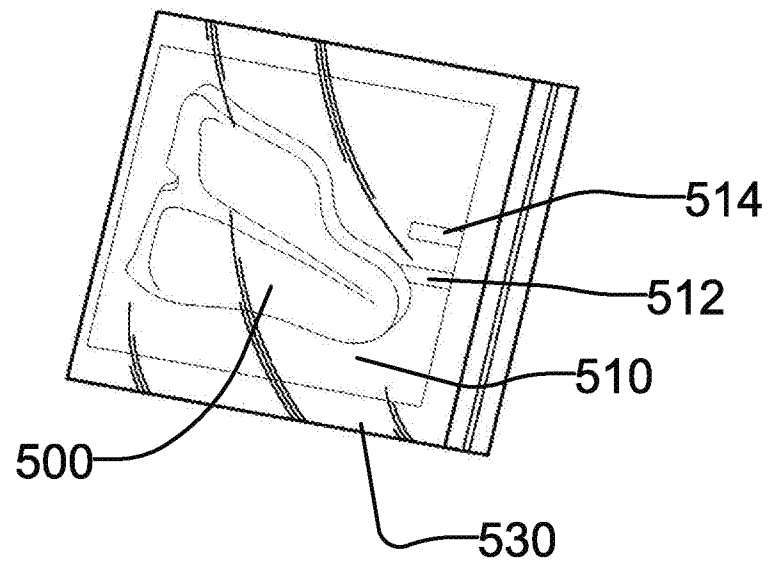
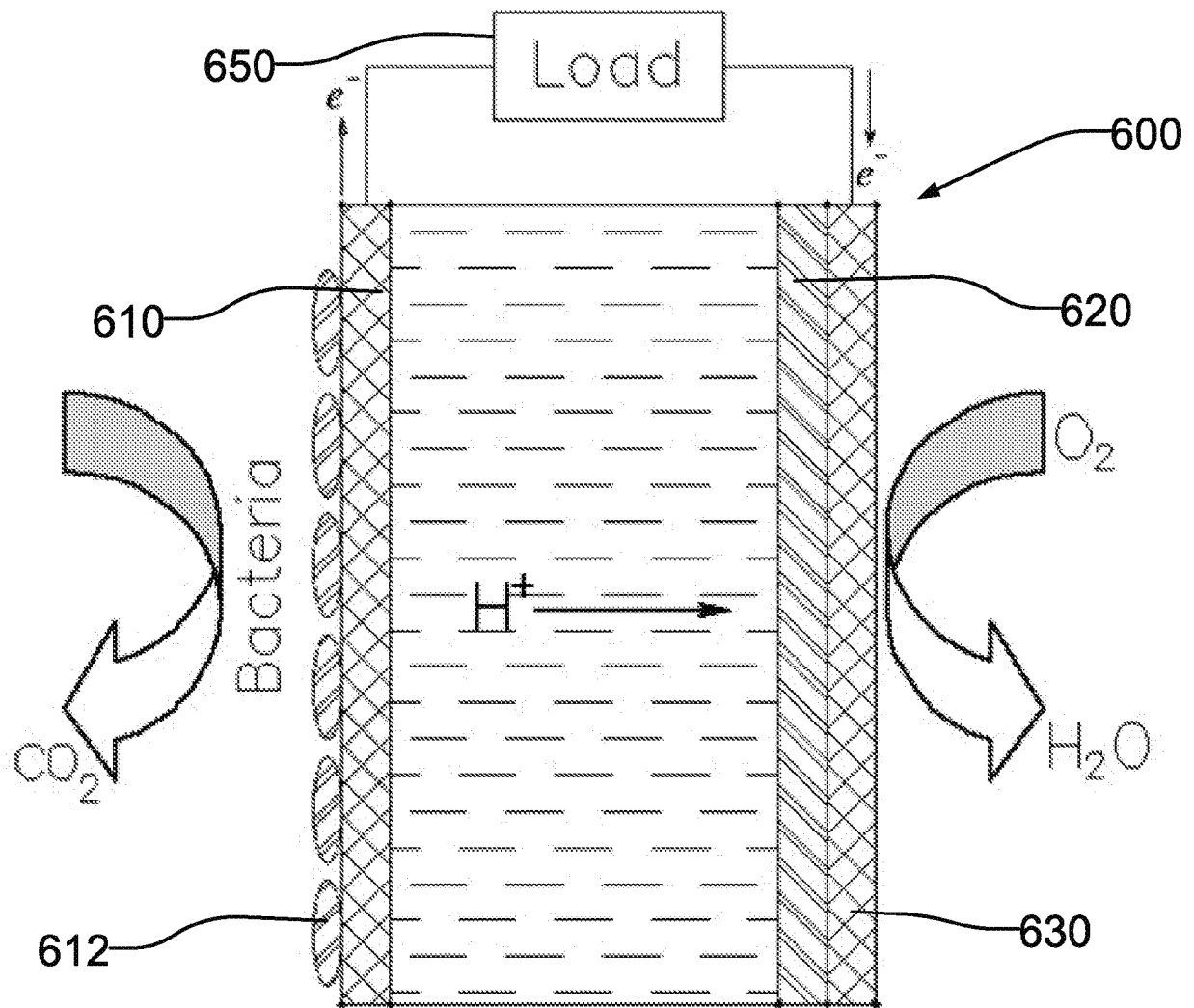
FIG. 6A*FIG. 6B*

FIG. 7A

6/7

**FIG. 8**

7/7

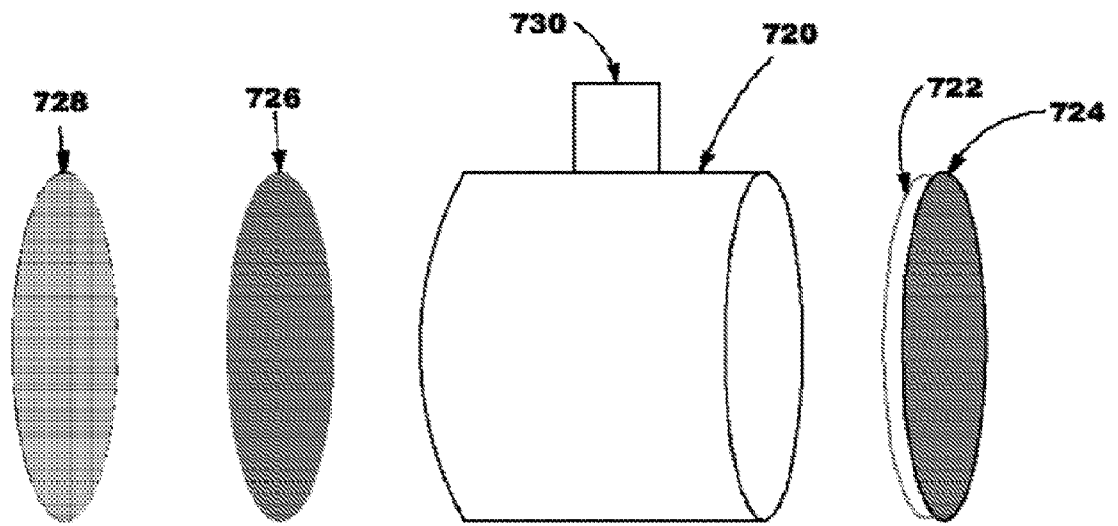


Fig. 9

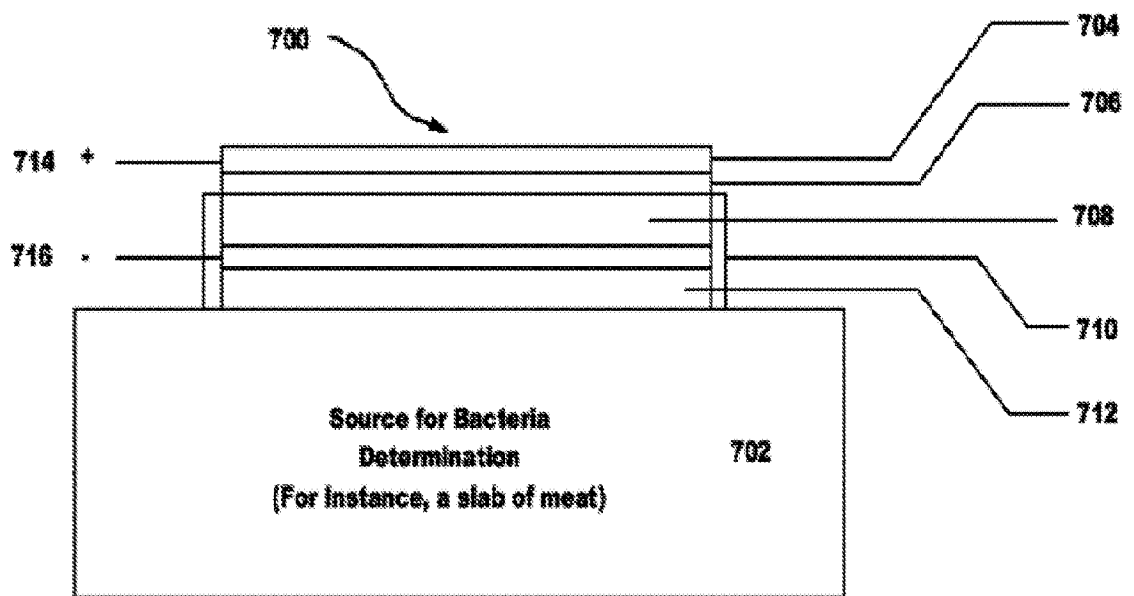


Fig. 10