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(71) Applicant *(for all designated States except US)*: **AKERMIN, INC.** [US/US]; 893 N. Warson Road, St. Louis, Missouri 63141 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **AKERS, Niki, L.** [US/US]; 7058 Stony Ridge Road, St. Louis, Missouri 63129 (US). **NELSON, David** [US/US]; 6970 Singingwood Lane, St. Louis, Missouri 63129 (US). **NELSON, Scott** [US/US]; 6970 Singingwood Lane, St. Louis, Missouri 63129 (US).

(74) Agents: **HENDRICKSON, Janet, S.** et al.; Senniger Powers, #1 Metropolitan Square, 16th Floor, St. Louis, Missouri 63102 (US).

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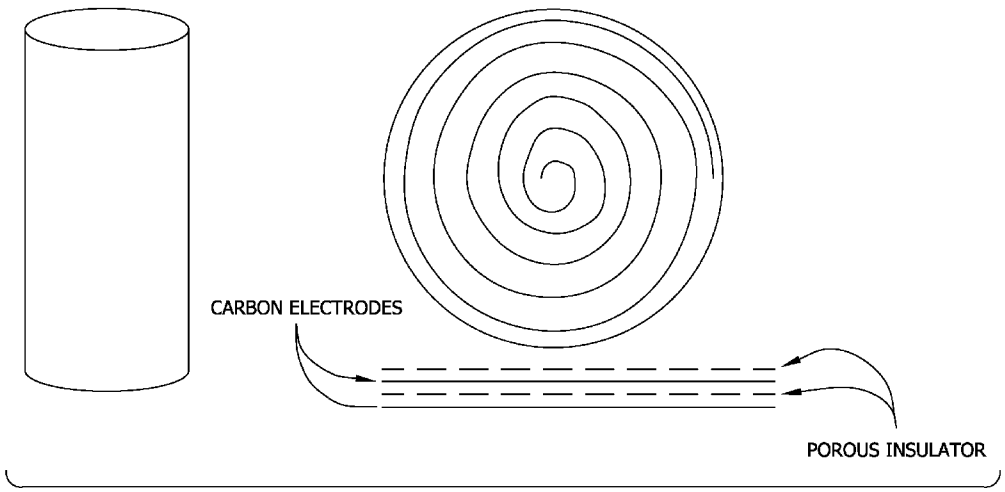
(54) **Title:** SPIRAL ELECTRODES IN BIOFUEL CELLS



(57) **Abstract:** Disclosed are novel bioanodes, biocathodes and biofuel cells having increased electrode area partially arising from the increased surface area of the bioanode or biocathode. The surface are of the bioanode or biocathode is increased due to the non-planar macroscopic shape relative to an otherwise identical macroscopically planar biocathode.

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SPIRAL ELECTRODES IN BIOFUEL CELLS

BACKGROUND OF THE INVENTION

[0001] The present invention is directed in general to biological enzyme-based fuel cells (a.k.a. biofuel cells) and their methods of manufacture and use. More specifically, the invention is directed to bioanodes, biocathodes and biofuel cells having spiral electrodes and their method of manufacture and use.

[0002] A biofuel cell is an electrochemical device in which energy derived from chemical reactions is converted to electrical energy by means of the catalytic activity of living cells and/or their enzymes. Biofuel cells generally use complex molecules to generate at the anode the hydrogen ions required to reduce oxygen to water, while generating free electrons for use in electrical applications. A bioanode is the electrode of the biofuel cell where electrons are released upon the oxidation of a fuel and a biocathode is the electrode where electrons and protons from the anode are used by the catalyst to reduce oxygen to water. Biofuel cells differ from the traditional fuel cell by the material used to catalyze the electrochemical reaction. Rather than using precious metals as catalysts, biofuel cells rely on biological molecules such as enzymes to carry out the reaction.

[0003] In U.S. Patent No. 6,063,517, Montemayor et al. describe a flexible, porous spiral cathode in ionic communication with a noble metal catalyst for use in fuel cells. This spiral cathode is paired with a planar anode that is in contact with the gaseous hydrogen fuel. In U.S. Patent Application Publication No. 2003/0021890, Marsacq et al. describe an electrode-membrane-electrode assembly on a cylindrical substrate that is made by successively depositing an electrode layer, a membrane layer and an electrode layer around the cylindrical substrate and then chemically or thermally eliminating the cylindrical substrate. Again the electrodes incorporate noble metals as catalysts. Thus, the fabrication of a spiral electrode for hydrogen fuel cells using noble metal catalysts is limited because the noble metal catalysts do not selectively catalyze either the oxidation or reduction reaction occurring in the fuel cell.

SUMMARY OF THE INVENTION

[0004] Among the various aspects of the present invention are novel bioanodes, biocathodes, and biofuel cells. One of the various aspects is a bioanode comprising an electron conductor, at least one anode enzyme, and an enzyme immobilization material. The anode

enzyme is capable of reacting with an oxidized form of the electron mediator and a fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator, the reduced form of the electron mediator is capable of releasing electrons to the electron conductor. The enzyme immobilization material is permeable to the fuel fluid and the electron mediator. The macroscopic shape of the bioanode is non-planar and the surface area of the bioanode is greater than that of an otherwise identical macroscopically planar bioanode.

[0005] Another of the aspects is a bioanode comprising an electron conductor, at least one anode enzyme, and an enzyme immobilization material. The anode enzyme is capable of reacting with an oxidized form of an electron mediator and a fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator, and the reduced form of the electron mediator is capable of releasing electrons to the electron conductor. The enzyme immobilization material comprises the electron mediator, and is permeable to the fuel fluid. The macroscopic shape of the bioanode is non-planar and the surface area of the bioanode is greater than that of an otherwise identical macroscopically planar bioanode.

[0006] Yet another of the aspects is a bioanode comprising an electron conductor, at least one anode enzyme, an enzyme immobilization material, and an electrocatalyst. The anode enzyme is capable of reacting with an oxidized form of the electron mediator and a fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator. The enzyme immobilization material is permeable to the fuel fluid and the electron mediator. The electrocatalyst is adjacent the electron conductor, and an oxidized form of the electrocatalyst is capable of reacting with the reduced form of the electron mediator to produce an oxidized form of the electron mediator and a reduced form of the electrocatalyst. The reduced form of the electrocatalyst is capable of releasing electrons to the electron conductor. The macroscopic shape of the bioanode is non-planar and the surface area of the bioanode is greater than that of an otherwise identical macroscopically planar bioanode.

[0007] Another aspect of the invention is a bioanode comprising an electron conductor, at least one anode enzyme, an enzyme immobilization material and an electrocatalyst. The anode enzyme is capable of reacting with an oxidized form of an electron mediator and a fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator. The enzyme immobilization material comprises the electron mediator and is permeable to the fuel fluid. The electrocatalyst is adjacent the electron conductor, and an oxidized form of the electrocatalyst is capable of reacting with the reduced form of the electron mediator to produce

an oxidized form of the electron mediator and a reduced form of the electrocatalyst, and the reduced form of the electrocatalyst is capable of releasing electrons to the electron conductor. The macroscopic shape of the bioanode is non-planar and the surface area of the bioanode is greater than that of an otherwise identical macroscopically planar bioanode.

[0008] A further aspect of the invention is a biocathode comprising an electron conductor, at least one cathode enzyme, and an enzyme immobilization material. The cathode enzyme is capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water. The enzyme immobilization material comprises an electrocatalyst, and is permeable to the oxidant, and an oxidized form of the electrocatalyst is capable of gaining electrons from the electron conductor to produce a reduced form of the electrocatalyst that is capable of reacting with an oxidized form of the electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst. The macroscopic shape of the biocathode is non-planar and the surface area of the biocathode is greater than that of an otherwise identical macroscopically planar biocathode.

[0009] Yet another aspect is a biocathode comprising an electron conductor, at least one cathode enzyme, and an enzyme immobilization material. The cathode enzyme is capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water. The enzyme immobilization material comprises the electron mediator, and is permeable to the oxidant. An oxidized form of the electron mediator is capable of gaining electrons from the electron conductor to produce a reduced form of the electron mediator. The macroscopic shape of the biocathode is non-planar and increases the surface area of the biocathode relative to a macroscopically planar biocathode.

[0010] Another aspect is a biocathode comprising an electron conductor, at least one cathode enzyme, and an enzyme immobilization material. The cathode enzyme is capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water. The enzyme immobilization material comprises the electron mediator and an electrocatalyst, and is permeable to the oxidant. An oxidized form of the electrocatalyst is capable of gaining electrons from the electron conductor to produce a reduced form of the electrocatalyst that is capable of reacting with an oxidized form of the electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst. The macroscopic shape of the biocathode is non-planar and the surface

area of the biocathode is greater than that of an otherwise identical macroscopically planar biocathode.

[0011] Yet another aspect is a biocathode comprising an electron conductor, at least one cathode enzyme, and an enzyme immobilization material. The cathode enzyme is capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water. The enzyme immobilization material comprising the electron mediator, and is permeable to the oxidant. An oxidized form of an electrocatalyst is capable of gaining electrons from the electron conductor to produce a reduced form of the electrocatalyst that is capable of reacting with an oxidized form of the electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst. The macroscopic shape of the biocathode is non-planar and the surface area of the biocathode is greater than that of an otherwise identical macroscopically planar biocathode.

[0012] A further aspect of the invention is a biofuel cell for generating electricity comprising a fuel fluid, an electron mediator, a bioanode as described above and a biocathode. Yet a further aspect is a biofuel cell for generating electricity comprising a fuel fluid, an electron mediator, a bioanode and a biocathode as described above. Yet another aspect is a biofuel cell for generating electricity comprising a fuel fluid, an electron mediator, a bioanode as described above and a biocathode as described above, wherein the bioanode and the biocathode are separated by a porous insulating material.

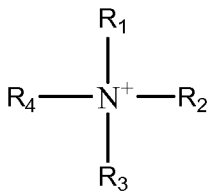
[0013] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the enzyme immobilization material comprises a micellar or inverted micellar structure.

[0014] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the enzyme immobilization material comprises a modified perfluoro sulfonic acid-PTFE copolymer.

[0015] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the electron conductor comprises a carbon-based material, a metallic conductor, a semiconductor, a metal oxide or a modified conductor; particularly, a carbon-based material.

[0016] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the electron conductor comprises carbon cloth, carbon paper, carbon screen printed electrodes, carbon black, carbon powder, carbon fiber, single-walled carbon nanotubes, double-walled carbon nanotubes, multi-walled carbon nanotubes, carbon nanotube arrays, diamond-coated conductors, glass carbon, mesoporous carbon, graphite, uncompressed graphite worms, delaminated purified flake graphite, high performance graphite, highly ordered pyrolytic graphite, pyrolytic graphite or polycrystalline graphite.

[0017] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the enzyme immobilization material is modified with a hydrophobic cation larger than NH_4^+; preferably, the hydrophobic cation comprises an ammonium-based cation, quaternary ammonium cation, alkyltrimethylammonium cation, organic cation, phosphonium cation, triphenylphosphonium, pyridinium cation, imidazolium cation, hexdecylpyridinium, ethidium, viologen, methyl viologen, benzyl viologen, bis(triphenylphosphine)iminium metal complex, bipyridyl metal complex, phenanthroline-based metal complex, $[Ru(bipyridine)_3]^{2+}$ or $[Fe(phenanthroline)_3]^{3+}$. In particular, the hydrophobic cation comprises a quaternary ammonium cation represented by formula 2



(2)

wherein R_1, R_2, R_3 and R_4 are independently hydrogen, hydrocarbyl, substituted hydrocarbyl or heterocyclo wherein at least one of R_1, R_2, R_3 and R_4 is other than hydrogen. In another embodiment, R_1, R_2, R_3 and R_4 are independently hydrogen, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl or tetradecyl wherein at least one of R_1, R_2, R_3 and R_4 is other than hydrogen. Alternatively, R_1, R_2, R_3 and R_4 are the same and are methyl, ethyl, propyl, butyl, pentyl or hexyl. Preferably, the quaternary ammonium cation of formula 1 is tetrabutylammonium, triethylhexylammonium or dodecyltrimethylammonium.

[0018] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the enzyme comprises an oxidoreductase; particularly, a glucose oxidase, alcohol-based oxidase or cholesterol-based oxidase.

[0019] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the enzyme comprises oxygen oxidoreductase having an optimum activity at a pH from about 6.5 to about 7.5; particularly, laccase, cytochrome C oxidase, bilirubin oxidase or peroxidase; more particularly, bilirubin oxidase.

[0020] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the electron mediator comprises a metalloprotein, a conjugated organic compound, a sugar, a sterol, a fatty acid or a coenzyme or substrate of an oxidase; particularly, wherein the oxidized form of the electron mediator comprises stellacyanin, bilirubin, glucose or cholesterol; more particularly, wherein the oxidized form of the electron mediator comprises bilirubin.

[0021] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the electrocatalyst for the electron mediator comprises organometallic cations with standard reduction potentials greater than +0.4 volts; particularly, wherein the electrocatalyst for the electron mediator comprises osmium, ruthenium, iron, nickel, rhodium, rhenium, or cobalt complexes; more particularly, wherein the reduced form of the electrocatalyst for the electron mediator comprises $Ru(phen)_3^{+2}$, $Fe(phen)_3^{+2}$, $Ru(bpy)_3^{+2}$, $Os(bpy)_3^{+2}$ or $Os(terpy)_3^{+2}$.

[0022] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the reduced form of the electrocatalyst for the electron mediator comprises $Ru(bpy)_3^{+2}$.

[0023] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the concentration of the electrocatalyst is from about 10 mM to about 3 M, more preferably from about 250 mM to about 2.25 M, still more preferably from about 500 mM to about 2 M, and most preferably from about 1.0 M to about 1.5 M.

[0024] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the oxidant comprises oxygen or peroxide; particularly, wherein the oxidant comprises oxygen.

[0025] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the fuel fluid comprises ammonia, methanol, ethanol, propanol, isobutanol, butanol and isopropanol, allyl alcohols, aryl alcohols, glycerol, propanediol, mannitol, glucuronate, aldehyde, carbohydrates, glucose, glucose-1, D-glucose, L-glucose, glucose-6-phosphate, lactate, lactate-6-phosphate, D-lactate, L-lactate, fructose, galactose-1, galactose, aldose, sorbose, mannose, glycerate, coenzyme A, acetyl Co-A, malate, isocitrate, formaldehyde, acetaldehyde, acetate, citrate, L-gluconate, beta-hydroxysteroid, alpha-hydroxysteroid, lactaldehyde, testosterone, gluconate, fatty acids, lipids, phosphoglycerate, retinal, estradiol, cyclopentanol, hexadecanol, long-chain alcohols, coniferyl-alcohol, cinnamyl-alcohol, formate, long-chain aldehydes, pyruvate, butanal, acyl-CoA, steroids, amino acids, flavin, NADH, $NADH_2$, NADPH, $NADPH_2$ or hydrogen; particularly, wherein the fuel fluid comprises methanol, ethanol or propanol; more particularly, wherein the fuel fluid comprises ethanol.

[0026] The present invention is still further directed to one or more of the previously described biofuel cells, bioanodes, biocathodes, and methods for generating electricity, wherein the modified perfluoro sulfonic acid-PTFE copolymer is modified with tetrabutylammonium bromide.

DESCRIPTION OF THE DRAWINGS

[0027] Figure 1 is a schematic of the cell case and a bioelectrode assembly in a circular form.

[0028] Figure 2 is a schematic of a design for a cellular telephone booster power source.

[0029] Figure 3 is a schematic of a design for a laptop power source.

[0030] Figure 4 is a set of graphs plotting the relationships between the running time, charging time and cell life and the percent of the power source occupied by the biofuel cell.

DETAILED DESCRIPTION OF THE INVENTION

[0031] Among the various aspects of the present invention is a biocathode or a bioanode having a non-planar macroscopic structure wherein the surface area of the biocathode or bioanode is increased relative to a biocathode or bioanode having a planar macroscopic structure. In various embodiments, the non-planar macroscopic shape of the biocathode or bioanode provides an electrode area at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more times greater than an otherwise identical biocathode or bioanode having a planar macroscopic shape. As the capacity of a biofuel cell is directly proportional to the surface area of the electrode, by bending, folding or winding the electrode, a higher surface area electrode is able to be placed into a constant volume container as compared to a planar, non-folded electrode material. Advantageously, the bioanodes and biocathodes of the present invention can be fabricated into assemblies that are alternating, bent, arcuate, folded, zigzag, spiral, coiled, or wound because the bioanode does not need to be physically separated from the biocathode. The bioanode and biocathode of an electrode assembly do not need to be physically separated because the enzyme contained in the bioanode selectively reacts with the fuel fluid and the enzyme contained in the biocathode selectively reacts with the oxidant to produce electricity.

[0032] In a further embodiment, the invention is directed to a bioelectrode assembly comprising a bioanode and a biocathode separated by a porous insulating material comprising an immobilized enzyme for use in an application wherein the surface area of the biocathode or bioanode is increased relative to a biocathode or bioanode having a planar macroscopic structure. Due to the selectivity of the enzymes immobilized in the bioanode and biocathode, the fuel fluid oxidized at the anode need not be separated from the fuel fluid reduced at the cathode. For example, a fuel fluid supersaturated with oxygen is used in the biofuel cell. Alternatively, the biofuel cell can comprise an air-breathing biocathode. In this embodiment, air is the oxidant and a portion of the biocathode is exposed to air, but in this case, the biocathode is not permeable to the fuel fluid.

[0033] The bioelectrode assemblies described above can be incorporated into a biofuel cell comprising a fuel fluid and an electron mediator. In a preferred embodiment, the bioanode comprises a PQQ-dependent enzyme and the biocathode comprises an electron mediator

present in a concentration sufficient to make the enzyme immobilization material conduct electrons.

[0034] In yet a further embodiment, the bioelectrode assembly of the present invention has increased enzyme stability. For use in a biocathode or a bioanode, the immobilization material forms a barrier that provides mechanical and chemical stability. Thus, the enzyme is stabilized for a longer period than previously known. In addition, for a biocathode, by incorporating a sufficient concentration of an electron mediator or electrocatalyst into the immobilization material, it acts as an electron mediator and electron transport through the immobilization material is maximized. For purposes of the present invention, an enzyme is "stabilized" if it retains at least about 75% of its initial catalytic activity while continuously generating electricity for at least about 30 days to about 730 days.

I. Biofuel Cell

[0035] Among the various aspects of the invention is a biofuel cell utilizing a fuel fluid to produce electricity via enzyme mediated redox reactions taking place at electrodes with immobilized enzymes therein. As in a standard electrochemical cell, the anode is the site for an oxidation reaction of a fuel fluid with a concurrent release of electrons. The electrons are directed from the anode through an electrical connector to some power consuming device. The electrons move through the device to another electrical connector, which transports the electrons to the biofuel cell's biocathode where the electrons are used to reduce an oxidant to produce water. In this manner, the biofuel cell of the present invention acts as an energy source (electricity) for an electrical load external thereto. To facilitate the fuel fluid's redox reactions, the electrodes comprise an electron conductor, an electron mediator, an electrocatalyst for the electron mediator, an enzyme, and an enzyme immobilization material.

[0036] In accordance with the invention, the electron mediator is a compound that can accept electrons or donate electrons. In a presently preferred biofuel cell, the oxidized form of the electron mediator reacts with the fuel fluid and the enzyme to produce the oxidized form of the fuel fluid and the reduced form of the electron mediator at the bioanode. Subsequently or concurrently, the reduced form of the electron mediator reacts with the oxidized form of the electrocatalyst to produce the oxidized form of the electron mediator and the reduced form of the electrocatalyst. The reduced form of the electrocatalyst is then oxidized at the bioanode and produces electrons to generate electricity. The redox reactions at the bioanode, except the

oxidation of the fuel fluid, can be reversible, so the enzyme, electron mediator and electrocatalyst are not consumed. Optionally, these redox reactions can be irreversible if an electron mediator and/or an electrocatalyst is added to provide additional reactant.

[0037] Alternatively, an electron conductor and an enzyme can be used wherein an electron mediator in contact with the bioanode is able to transfer electrons between its oxidized and reduced forms at unmodified electrodes. If the electron mediator is able to transfer electrons between its oxidized and reduced forms at an unmodified bioanode, the subsequent reaction between the electrocatalyst and the electron mediator is not necessary and the electron mediator itself is oxidized at the bioanode to produce electrons and thus, electricity.

[0038] At the biocathode, electrons originating from the bioanode flow into the biocathode's electron conductor. There, the electrons combine with an oxidized form of an electrocatalyst, which is in contact with the electron conductor. This reaction produces a reduced form of the electrocatalyst, which in turn reacts with an oxidized form of an electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst. Next, the reduced form of the electron mediator reacts with an oxidized form of the oxidant to produce an oxidized form of the electron mediator and water. In one embodiment, an enzyme immobilization material permeable to the oxidant is present, which comprises the electrocatalyst and, optionally, the electron mediator, and which is capable of immobilizing and stabilizing the enzyme.

[0039] In an alternative embodiment of the biocathode, there is no electrocatalyst present. In this embodiment, the electrons combine with an oxidized form of the electron mediator to produce a reduced form of the electron mediator. Then, the reduced form of the electron mediator reacts with an oxidized form of an oxidant to produce an oxidized form of the electron mediator and water. In one embodiment, an enzyme immobilization material permeable to the oxidant is present, which optionally comprises the electron mediator, and which is capable of immobilizing and stabilizing the enzyme.

[0040] The biofuel cell of the present invention comprises a biocathode and/or a bioanode. Generally, the bioanode comprises elements that effect the oxidation of fuel fluid whereby electrons are released and directed to an external electrical load. The resulting electrical current powers the electrical load, with electrons being subsequently directed to a biocathode where an oxidant is reduced and water is produced.

A. Bioelectrode Assembly

[0041] The bioelectrode assembly of the present invention comprises a bioanode and a biocathode separated by a porous insulating material. Contacts can be placed at many points along the bioanode and biocathode to carry power to the load (e.g., the device that is using the biofuel cell as a power source). As described above, the bioanode and biocathode electrode assembly can be configured into a variety of shapes because a physical separation of the anode compartment from the cathode compartment (e.g., a salt bridge or polymer electrolyte membrane) is not required. Such a physical separation is not required because of the selectivity of the bioanode enzyme for reaction with the fuel fluid and the selectivity of the biocathode enzyme for reaction with the oxidant. However, in order to prevent electrical contact of the bioanode with the biocathode, an insulating material does separate the bioanode and the biocathode, while keeping the bioanode and biocathode connected ionically (e.g., through the fuel fluid). The shapes and components of the bioelectrode assembly are described below.

[0042] In many of the various embodiments, the bioelectrode assembly comprises a bioanode having a macroscopic shape that is non-planar and the surface area of the bioanode is greater than that of an otherwise identical macroscopically planar bioanode. A macroscopically planar bioanode or biocathode does not have a deviation in the z-axis of more than about 0.01 cm, 0.02 cm, 0.03 cm, 0.04 cm, 0.05 cm, 0.06 cm, 0.07 cm, 0.08 cm, 0.09 cm, 0.1 cm, 0.12 cm, 0.14 cm, 0.16 cm, 0.18 cm, or 0.2 cm, if the main plane of the electrode is in the x- and y-axes.

[0043] In other embodiments, the bioelectrode assembly of the present invention comprises a biocathode having a macroscopic shape that is non-planar and the surface area of the biocathode is greater than that of an otherwise identical macroscopically planar biocathode.

[0044] Preferably, the bioelectrode assembly of the present invention comprises a bioanode having a macroscopic shape that is non-planar and the surface area of the bioanode is greater than that of an otherwise identical macroscopically planar bioanode and a biocathode having a macroscopic shape that is non-planar and the surface area of the biocathode is greater than that of an otherwise identical macroscopically planar biocathode.

[0045] Advantageously, the surface area of the bioanode and/or biocathode is maximized by bending, folding or winding the bioanode and/or biocathode. As a result of the increased surface area of the bioanode and/or biocathode, the capacity of a biofuel cell is

increased while the higher capacity electrode assembly still fits in a container with the same volume. In contrast, a planar, non-folded electrode assembly of a bioanode and biocathode would require a larger volume container in order to increase the capacity of the electrode assembly.

[0046] Consequently, preferred shapes for the electrode assembly are those that have a substantially non-planar configuration. For example, the bioanode and biocathode or the bioelectrode assembly can be manipulated in order to form shapes that are alternating, bent, arcuate, folded, zigzag, spiral, coiled, wound, or combinations thereof. The shape of the bioanode, biocathode or bioelectrode assembly is chosen to maximize the surface area of the bioanode, biocathode or bioelectrode assembly while still allowing it to have a small volume in order to increase the capacity of the biofuel cell.

[0047] Generally, the bioelectrode assembly of a bioanode and a biocathode can be prepared by using a bioanode as described in U.S. Patent Application Serial No. 10/617,452 (published as U.S. Patent Application Publication No. 2004/0101741), separating the bioanode from a biocathode using a porous insulating material described below wherein the biocathode is described in U.S. Patent Application Serial No. 10/931,147 (published as U.S. Patent Application Publication No. 2005/0095466). Once the bioelectrode assembly comprising a bioanode and biocathode separated by a porous insulating material is made, this bioelectrode assembly can be manipulated to form a geometry having a macroscopic non-planar geometry. For example, these non-planar geometries can be alternating, bent, arcuate, folded, zigzag, spiral, coiled, wound, or combinations thereof.

[0048] In other bioelectrode assemblies of the invention, the bioanode can be separated from the biocathode by a porous insulating material and the bioanode can then be manipulated to form a geometry having a macroscopic non-planar geometry (*e.g.*, alternating, bent, arcuate, folded, zigzag, spiral, coiled, wound, or combinations thereof). Alternatively, the bioanode can be separated from the biocathode by a porous insulating material and the biocathode can then be manipulated to form a geometry having a macroscopic non-planar geometry (*e.g.*, alternating, bent, arcuate, folded, zigzag, spiral, coiled, wound, or combinations thereof).

[0049] When a bioelectrode assembly comprising a bioanode and a biocathode is fabricated in a shape that maximizes surface area as described above, a separation material that is porous and insulating is used to separate the bioanode from the biocathode to limit the potential for short circuiting between the bioanode and biocathode while allowing the chemical

reactants to pass through it. This separation material has a porosity such that the chemical reactants (*e.g.*, fuel fluid, oxidant, optionally, the electron mediator and optionally, the electrocatalyst) freely pass through the material and the mass transport of these chemical reactants is not substantially altered as compared to their mass transport in a biofuel cell not containing the separation material. Further, the separation material is insulating; thus, it is able to provide an electric separation between the bioanode and biocathode.

[0050] There are a variety of materials that can be used as the separation material. For example, meshes fabricated from polypropylene, polyethylene, nylon, polyvinyl chloride (PVC), polytetrafluoroethylene (PTFE), polyester, ethylene vinyl acetate (EVA), thermoplastic polymers (e.g., sold under the tradename Santoprene®), styrenic block copolymers (e.g., Kraton® polymers) and combinations thereof. The meshes described have a thickness from about 0.006 inch to about 0.2 inch (about 0.15 mm to about 5 mm); preferably, from about 0.006 inch to about 0.1 inch (about 0.15 mm to about 2.5 mm); more preferably, from about 0.006 inch to about 0.05 inch (about 0.15 mm to about 1.3 mm). These meshes preferably have a nominal open area from about 15% to about 70%; preferably, from about 50% to about 70%.

[0051] In various embodiments of the present invention, the shape of the bioanode, biocathode or bioelectrode assembly can be those described above. In order to simplify the calculations for the area and capacity of the bioelectrode assembly, a circular configuration for the assembly was used (See figure 1). Various parameters can be calculated from the following formulae.

Electrode area (cm^2)	$A = 2\pi d \cdot \sum_{r=0}^{\left\lfloor \frac{d-t}{2t} \right\rfloor} (.025 + .05r)$	(3)
Power (W)	$P = A \cdot \sigma_P$	(4)
Maximum current (A)	$I_{max} = \frac{P}{v_{op}}$	(5)
Fuel volume (cm^3)	$V = \pi \left(\frac{d}{2} \right)^2 \cdot l \cdot \alpha$	(6)
Capacity (Wh)	$E = V \cdot \rho_E$	(7)

Charge (Ah)	$C = \frac{E}{v_{op}}$ (8)
Minimum running time (h)	$T = \frac{E}{I_{max} \cdot v_{op}}$ (9)

[0052] For formulae 3 to 9, r is the radius of the spiral in cm, d is the diameter of the spiral in cm, l is the length of the spiral in cm, t is the thickness of an electrode stack (assumed to be 0.05 cm as described below), v_{op} is the operating voltage and equals 1.5 V for alkaline batteries, α is the fuel to volume ratio, σ_p is the power density of the cell in W/cm^2 (assumed to be 0.010 W/cm^2 as described below), and ρ_E is the energy density in Wh/cm^3 (assumed to be 1.87 Wh/cm^3 as described below). Using the equations above and values for the variables, the following quantities can be calculated: A (the electrode area in cm^2), P (the power in W), I_{max} (the maximum current in A), V (the fuel volume in cm^3), E (the capacity in Wh), C (the charge in Ah), and T (the running time in h).

[0053] It is assumed that the battery's metal container has negligible thickness and thus, the electrode extends to the physical limits of the battery. Further, the sustained power density is estimated at 0.010 W/cm^2 from experimental data. The energy density of 1.87 Wh/mL is estimated by starting with the published energy density of ethanol of 6.1 Wh/mL, then dividing this value by a factor of 3 because the oxidation of ethanol at the bioanode releases 4 electrons out of 12 total electrons available. Then, the value is further reduced to estimate the resistance of the system and the electrical losses inherent in the system to arrive at a value of 1.87 Wh/mL that is used throughout the calculations described. It is further assumed that the carbon electrodes have negligible thickness. If the porous insulator placed between the carbon layers is 0.25 mm thick, the thickness of one electrode stack is 0.5 mm thick ((+)carbon, spacer, (-)carbon, spacer). In addition it is assumed that the electrode stack occupies half of the available volume, and that the other half of the volume of the cell is occupied by fuel solution ($\alpha = 0.5$). Calculations for the spiral electrodes described above are presented in more detail in examples 2 and 3 below.

1. Biocathode

[0054] The biocathode in accordance with this invention comprises an electron conductor, an enzyme which is immobilized in an enzyme immobilization material, an electron

mediator, and an electrocatalyst wherein the macroscopic shape of the biocathode is non-planar and the non-planar biocathode has an increased surface area relative to a macroscopically planar biocathode. In one embodiment, these components are adjacent to one another, meaning they are physically or chemically connected by appropriate means.

a. Electron Conductor

[0055] The electron conductor is a substance that conducts electrons. The electron conductor can be organic or inorganic in nature as long as it is able to conduct electrons through the material. The electron conductor can be a carbon-based material, stainless steel, stainless steel mesh, a metallic conductor, a semiconductor, a metal oxide, or a modified conductor. In a preferred embodiment, the electron conductor is carbon paper.

[0056] Particularly suitable electron conductors are carbon-based materials. Exemplary carbon-based materials are carbon cloth, carbon paper, carbon screen printed electrodes, carbon paper (Toray), carbon paper (ELAT), carbon paper (Ballard AvCarb™), carbon black (Vulcan XC-72, E-tek), carbon black, carbon powder, carbon fiber, single-walled carbon nanotubes, double-walled carbon nanotubes, multi-walled carbon nanotubes, carbon nanotubes arrays, diamond-coated conductors, glassy carbon and mesoporous carbon. In addition, other exemplary carbon-based materials are graphite, uncompressed graphite worms, delaminated purified flake graphite (Superior® graphite), high performance graphite and carbon powders (Formula BT™, Superior® graphite), highly ordered pyrolytic graphite, pyrolytic graphite and polycrystalline graphite. A preferred electron conductor is a sheet of carbon paper (Ballard AvCarb™).

[0057] In a further embodiment, the electron conductor can be made of a metallic conductor. Suitable electron conductors can be prepared from gold, platinum, iron, nickel, copper, silver, stainless steel, mercury, tungsten, and other metals suitable for electrode construction. In addition, electron conductors which are metallic conductors can be constructed of nanoparticles made of cobalt, carbon, and other suitable metals. Other metallic electron conductors can be silver-plated nickel screen printed electrodes.

[0058] In addition, the electron conductor can be a semiconductor. Suitable semiconductor materials include silicon and germanium, which can be doped with other

elements. The semiconductors can be doped with phosphorus, boron, gallium, arsenic, indium or antimony, or a combination thereof.

[0059] Other electron conductors can be metal oxides, metal sulfides, main group compounds (i.e., transition metal compounds), and materials modified with electron conductors. Exemplary electron conductors of this type are nanoporous titanium oxide, tin oxide coated glass, cerium oxide particles, molybdenum sulfide, boron nitride nanotubes, aerogels modified with a conductive material such as carbon, solgels modified with conductive material such as carbon, ruthenium carbon aerogels, and mesoporous silicas modified with a conductive material such as carbon.

b. Electron Mediators

[0060] The electron mediator is a compound that can accept or donate electron(s). Stated another way, the electron mediator has an oxidized form that can accept electron(s) to form the reduced form, wherein the reduced form can also donate electron(s) to produce the oxidized form. The electron mediator is a compound that can diffuse into the immobilization material and/or be incorporated into the immobilization material.

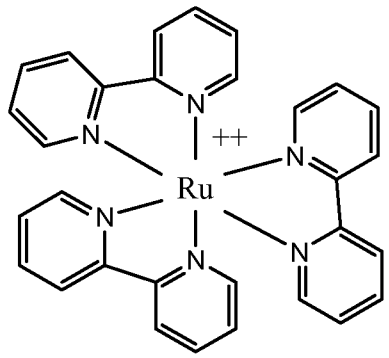
[0061] In one embodiment, the diffusion coefficient of the electron mediator is maximized. Stated another way, mass transport of the reduced form of the electron mediator is as fast as possible. A fast mass transport of the electron mediator allows for a greater current and power density of the biofuel cell in which it is employed.

[0062] The biocathode's electron mediator can be a protein such as stellacyanin, a protein byproduct such as bilirubin, a sugar such as glucose, a sterol such as cholesterol, a fatty acid, or a metalloprotein. The electron mediators can also be a coenzyme or substrate of an oxidase. In one preferred embodiment, the electron mediator at the biocathode is bilirubin.

c. Electrocatalyst for an Electron Mediator

[0063] Generally, the electrocatalyst is a substance that facilitates the release of electrons at the electron conductor by reducing the standard reduction potential of the electron mediator.

[0064] Generally, electrocatalysts according to the invention are organometallic cations with standard reduction potentials greater than +0.4 volts. Exemplary electrocatalysts are transition metal complexes, such as osmium, ruthenium, iron, nickel, rhodium, rhenium, and cobalt complexes. Preferred organometallic cations using these complexes comprise large organic aromatic ligands that allow for large electron self exchange rates. Examples of large organic aromatic ligands include derivatives of 1,10-phenanthroline (phen), 2,2'-bipyridine (bpy) and 2,2',2"-terpyridines (terpy), such as $Ru(phen)_3^{+2}$, $Fe(phen)_3^{+2}$, $Ru(bpy)_3^{+2}$, $Os(bpy)_3^{+2}$, and $Os(terpy)_3^{+2}$. In a preferred embodiment, the electrocatalyst is a ruthenium compound. Most preferably, the electrocatalyst at the biocathode is $Ru(bpy)_3^{+2}$ (represented by Formula 1).



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[0065] The electrocatalyst is present in a concentration that facilitates the efficient transfer of electrons. Preferably, the electrocatalyst is present at a concentration that makes the enzyme immobilization material conduct electrons. Particularly, the electrocatalyst is present at a concentration of from about 10 mM to about 3 M, more preferably from about 250 mM to about 2.25 M, still more preferably from about 500 mM to about 2 M, and most preferably from about 1.0 M to about 1.5 M.

d. Enzyme

[0066] In accordance with the invention, an enzyme reduces an oxidant at the biocathode. Generally, naturally-occurring enzymes, man-made enzymes, artificial enzymes and modified naturally-occurring enzymes can be utilized. In addition, engineered enzymes that have been engineered by natural or directed evolution can be used. Stated another way, an

organic or inorganic molecule that mimics an enzyme's properties can be used in an embodiment of the present invention.

[0067] Specifically, exemplary enzymes for use in a biocathode are oxidoreductases. Potential oxidoreductases include laccases and oxidases, such as glucose oxidase, alcohol-based oxidases, and cholesterol-based oxidases. In a preferred embodiment, the enzyme is a peroxidase or oxygen oxidoreductase, which catalyze the reduction hydrogen peroxide and oxygen, respectively. Exemplary oxygen oxidoreductases include laccase, cytochrome c oxidase, bilirubin oxidase and peroxidase. More preferably, the enzyme is an oxygen oxidoreductase having an optimum activity at a pH between about 6.5 and about 7.5. An oxidoreductase having an optimum activity at a pH from about 6.5 to about 7.5 is advantageous for applications directed to a physiological environment, such as a plant or a human or animal body. Most preferably, the enzyme is a bilirubin oxidase.

e. Enzyme Immobilization Material

[0068] An enzyme immobilization material is utilized in the biofuel cell at the bioanode and/or the biocathode. In one embodiment, the bioanode's enzyme immobilization material is permeable to the fuel fluid and immobilizes and stabilizes the enzyme. The immobilization material is permeable to the fuel fluid so the oxidation reaction of the fuel at the bioanode can be catalyzed by the immobilized enzyme.

[0069] Generally, an enzyme is used to catalyze redox reactions at the biocathode and/or the bioanode. In an bioanode and/or biocathode according to this invention, an enzyme is immobilized in an enzyme immobilization material that both immobilizes and stabilizes the enzyme. Typically, a free enzyme in solution loses its catalytic activity within a few hours to a few days, whereas a properly immobilized and stabilized enzyme can retain its catalytic activity for at least about 30 days to about 730 days. The retention of catalytic activity is defined as the enzyme having at least about 75% of its initial activity while continuously producing electricity, which can be measured by chemiluminescence, electrochemical, UV-Vis, radiochemical, or fluorescence assay.

[0070] An immobilized enzyme is an enzyme that is physically confined in a certain region of the enzyme immobilization material while retaining its catalytic activity. There are a variety of methods for enzyme immobilization, including carrier-binding, cross-linking and

entrapping. Carrier-binding is the binding of enzymes to water-insoluble carriers. Cross-linking is the intermolecular cross-linking of enzymes by bifunctional or multifunctional reagents. Entrapping is incorporating enzymes into the lattices of a semipermeable material. The particular method of enzyme immobilization is not critically important, so long as the enzyme immobilization material (1) immobilizes the enzyme, (2) stabilizes the enzyme, and (3) is permeable to the fuel fluid or oxidant.

[0071] With reference to the enzyme immobilization material's permeability to the fuel fluid or oxidant and the immobilization of the enzyme, in one embodiment, the material is permeable to a compound that is smaller than an enzyme. Stated another way, the enzyme immobilization material allows the movement of the fuel fluid or oxidant compound through it so the compound can contact the enzyme. The enzyme immobilization material can be prepared in a manner such that it contains internal pores, channels, openings or a combination thereof, which allow the movement of the compound throughout the enzyme immobilization material, but which constrain the enzyme to substantially the same space within the enzyme immobilization material. Such constraint allows the enzyme to retain its catalytic activity. In one preferred embodiment, the enzyme is confined to a space that is substantially the same size and shape as the enzyme, wherein the enzyme retains substantially all of its catalytic activity. The pores, channels, or openings have physical dimensions that satisfy the above requirements and depend on the size and shape of the specific enzyme to be immobilized.

[0072] In one embodiment, the enzyme is preferably located within a pore of the enzyme immobilization material and the compound travels in and out of the enzyme immobilization material through transport channels. The relative size of the pores and transport channels can be such that a pore is large enough to immobilize an enzyme, but the transport channels are too small for the enzyme to travel through them. Further, a transport channel preferably has a diameter of at least about 10 nm. In still another embodiment, the pore diameter to transport channel diameter ratio is at least about 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, 10:1 or more. In yet another embodiment, preferably, a transport channel has a diameter of at least about 10 nm and the pore diameter to transport channel diameter ratio is at least about 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, 10:1 or more.

[0073] With respect to the stabilization of the enzyme, the enzyme immobilization material provides a chemical and mechanical barrier to prevent or impede enzyme

denaturation. To this end, the enzyme immobilization material physically confines the enzyme, preventing the enzyme from unfolding. The process of unfolding an enzyme from a folded three-dimensional structure is one mechanism of enzyme denaturation. In one embodiment, the immobilization material, preferably, stabilizes the enzyme so that the enzyme retains its catalytic activity during continuous electricity generation for at least about 30 days to about 730 days. The retention of catalytic activity is defined by the number of days that the enzyme retains at least about 75% of its initial activity while continuously producing electricity. The enzyme activity can be measured by chemiluminescence, electrochemical, UV-Vis, radiochemical or fluorescence assay wherein the intensity of the property is measured at an initial time. Typically, a fluorescence assay is used to measure the enzyme activity. A free enzyme in solution loses its catalytic activity within hours to a few days. Thus, the immobilization of the enzyme provides a significant advantage in stability. In another embodiment, preferably, the immobilized enzyme retains at least about 75% of its initial catalytic activity during continuous electricity generation for at least about 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 330, 365, 400, 450, 500, 550, 600, 650, 700, 730 days or more, preferably retaining at least about 80%, 85%, 90%, 95% or more of its initial catalytic activity for at least about 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 330, 365, 400, 450, 500, 550, 600, 650, 700, 730 days or more.

[0074] In one embodiment, the enzyme immobilization material is a non-naturally occurring colloidal material. In another embodiment, the enzyme immobilization material is an acellular colloidal material, such as liposomes. An acellular material is not made up of and does not contain cells. A colloidal material is a substance that consists of particles dispersed throughout another substance which are too small for resolution with an ordinary light microscope but are incapable of passing through a semipermeable membrane. Furthermore, a colloidal material is a substance consisting of particles substantially larger than atoms or ordinary molecules but too small to be visible to the unaided eye. They can range in size from about 10^{-7} to 10^{-3} centimeters and are linked or bonded together in a variety of ways.

[0075] In yet another embodiment, the enzyme immobilization material has a micellar or inverted micellar structure. Generally, the molecules making up a micelle are amphipathic, meaning they contain a polar, hydrophilic group and a nonpolar, hydrophobic group. The molecules can aggregate to form a micelle, where the polar groups are on the surface of the aggregate and the hydrocarbon, nonpolar groups are sequestered inside the aggregate. Inverted

micelles have the opposite orientation of polar groups and nonpolar groups. The amphipathic molecules making up the aggregate can be arranged in a variety of ways so long as the polar groups are in proximity to each other and the nonpolar groups are in proximity to each other. Also, the molecules can form a bilayer with the nonpolar groups pointing toward each other and the polar groups pointing away from each other. Alternatively, a bilayer can form wherein the polar groups can point toward each other in the bilayer, while the nonpolar groups point away from each other.

[0076] Generally, the micellar or inverted micellar enzyme immobilization material can be a polymer, a ceramic, a liposome, or any other material made of molecules that form a micellar or inverted micellar structure. Exemplary micellar or inverted micellar enzyme immobilization materials are perfluoro sulfonic acid-polytetrafluoro ethylene (PTFE) copolymer (or perfluorinated ion exchange polymer)(Nafion® or Flemion®), modified perfluoro sulfonic acid-polytetrafluoro ethylene (PTFE) copolymer (or modified perfluorinated ion exchange polymer)(modified Nafion® or modified Flemion®), polysulfone, micellar polymers, poly(ethylene oxide) based block copolymers, polymers formed from microemulsion and/or micellar polymerization and copolymers of alkyl methacrylates, alkyl acrylates, and styrenes. Other exemplary micellar or inverted micellar immobilization materials are ceramics, sodium bis(2-ethylhexyl)sulfosuccinate, sodium dioctylsulfosuccinate, lipids, phospholipids, sodium dodecyl sulfate, decyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, (4-[(2-hydroxyl-1-naphthalenyl)azo]benzenesulfonic acid monosodium salt), linoleic acids, linolenic acids, colloids, liposomes and micelle networks.

[0077] In one preferred embodiment, the micellar enzyme immobilization material is a modified perfluoro sulfonic acid-PTFE copolymer (or modified perfluorinated ion exchange polymer)(modified Nafion® or modified Flemion®) membrane. The perfluorinated ion exchange polymer membrane is modified with a hydrophobic cation that is larger than the ammonium (NH_4^+) ion. The hydrophobic cation serves the dual function of (1) dictating the membrane's pore size and (2) acting as a chemical buffer to help maintain the pore's pH level, both of which further efforts to stabilize the enzyme.

[0078] With regard to the first function of the hydrophobic cation, mixture-casting a perfluoro sulfonic acid-PTFE copolymer (or perfluorinated ion exchange polymer) with a hydrophobic cation to produce a modified perfluoro sulfonic acid-PTFE copolymer (or

modified perfluorinated ion exchange polymer)(Nafion® or Flemion®) membrane provides an enzyme immobilization material wherein the pore size is dependent on the size of the hydrophobic cation. Accordingly, the larger the hydrophobic cation, the larger the pore size. This function of the hydrophobic cation allows the pore size to be made larger or smaller to fit a specific enzyme by varying the size of the hydrophobic cation.

[0079] Regarding the second function of the hydrophobic cation, the properties of the perfluoro sulfonic acid-PTFE copolymer (or perfluorinated ion exchange polymer) membrane are altered by exchanging the hydrophobic cation for protons as the counterion to the $-SO_3^-$ groups on the perfluoro sulfonic acid-PTFE copolymer (or perfluorinated ion exchange polymer) membrane. This change in counterion provides a buffering effect on the pH because the hydrophobic cation has a much greater affinity for the $-SO_3^-$ sites than protons do. This buffering effect of the membrane causes the pH of the pore to remain substantially unchanged with changing solution pH; stated another way, the pH of the pore resists changes in the solution's pH. In addition, the membrane provides a mechanical barrier, which further protects the immobilized enzymes.

[0080] The following table demonstrates the buffering effect of the modified perfluoro sulfonic acid-PTFE copolymer membrane. The values represent the number of available exchange sites for protons per gram of modified perfluoro sulfonic acid-PTFE copolymer membrane; as the number of exchange sites available to protons decreases, the buffering capacity of the membrane toward the immobilized enzyme increases. The membrane abbreviations designate the following membranes: NH_4Br is an ammonium bromide-modified Nafion® membrane, TMABr is a tetramethylammonium bromide-modified Nafion® membrane, TEABr is a tetraethylammonium bromide-modified Nafion® membrane, TpropABr is a tetrapropylammonium bromide-modified Nafion® membrane, TBABr is a tetrabutylammonium bromide-modified Nafion® membrane, and TpentABr is a tetrapentylammonium bromide-modified Nafion® membrane.

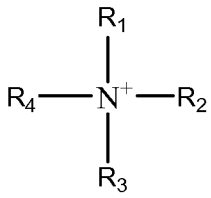
Membrane	**Mixture-Cast ($x10^{-6}$ mole/g)**	**Salt-Extracted ($x10^{-6}$ mole/g)**
Nafion®	907 ± 68	----
NH_4Br	521 ± 74	591 ± 95

TMABr	171 ± 19	458 ± 27
TEABr	157 ± 4	185 ± 22
TPropABr	133 ± 6	138 ± 77
TBABr	8.68 ± 2.12	96 ± 23
TPentABr	2.71 ± 0.6	1.78 ± 1.66

[0081] In order to prepare a modified perfluoro sulfonic acid-PTFE copolymer (or perfluorinated ion exchange polymer) membrane, the first step is to cast a suspension of perfluoro sulfonic acid-PTFE copolymer (or perfluorinated ion exchange polymer), particularly Nafion®, with a solution of the hydrophobic cations to form a membrane. After extracting the excess hydrophobic cations and their salts from the original membrane, the membrane is re-cast. Upon re-casting, the membrane contains the hydrophobic cations in association with the $-SO_3^-$ sites of the perfluoro sulfonic acid-PTFE copolymer (or perfluorinated ion exchange polymer) membrane.

[0082] In order to make more stable and reproducible quaternary ammonium salt-treated Nafion® membranes, the excess bromide salts must be removed from the casting solution. This salt-extracted membrane is formed by re-casting the mixture-cast membranes after the excess quaternary ammonium bromide and HBr salts have been extracted from the original membranes. Salt extraction of membranes retains the presence of the quaternary ammonium cations at the sulfonic acid exchange sites, but eliminates complications from excess salt that may be trapped in the pore or may cause voids in the equilibrated membrane. The chemical and physical properties of the salt-extracted membranes have been characterized by voltammetry, ion exchange capacity measurements, and fluorescence microscopy before enzyme immobilization. Exemplary hydrophobic cations are ammonium-based cations, quaternary ammonium cations, alkyltrimethylammonium cations, alkyltriethylammonium cations, organic cations, phosphonium cations, triphenylphosphonium, pyridinium cations, imidazolium cations, hexdecylpyridinium, ethidium, viologens, methyl viologen, benzyl viologen, bis(triphenylphosphine)iminium, metal complexes, bipyridyl metal complexes, phenanthroline-based metal complexes, $[Ru(bipyridine)_3]^{2+}$ and $[Fe(phenanthroline)_3]^{3+}$.

[0083] In one preferred embodiment, the hydrophobic cations are ammonium-based cations. In particular, the hydrophobic cations are quaternary ammonium cations. In another embodiment, the quaternary ammonium cations are represented by formula (2):



(2)

wherein R_1, R_2, R_3, and R_4 are independently hydrogen, hydrocarbyl, substituted hydrocarbyl, or heterocyclo wherein at least one of R_1, R_2, R_3, and R_4 is other than hydrogen. In a further embodiment, preferably, R_1, R_2, R_3, and R_4 are independently hydrogen, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl or tetradecyl wherein at least one of R_1, R_2, R_3, and R_4 is other than hydrogen. In still another embodiment, R_1, R_2, R_3, and R_4 are the same and are methyl, ethyl, propyl, butyl, pentyl or hexyl. In yet another embodiment, preferably, R_1, R_2, R_3, and R_4 are butyl. Preferably, the quaternary ammonium cation is tetrabutylammonium, triethylhexylammonium or dodecyl trimethylammonium.

[0084] Mixture-cast films of quaternary ammonium salts or surfactants (e.g., TBAB, triethylhexylammonium bromide, trimethyldodecylammonium bromide, and phenyltrimethylammonium bromide) and Nafion® have increased the mass transport of small analytes through the films and decreased the selectivity of the enzyme immobilization membrane against anions. These enzyme immobilization membranes have very similar conductivities as unmodified Nafion, but they have a much higher preference to the quaternary ammonium bromide than to the proton, as shown by titrating the number of available exchange sites to protons in the enzyme immobilization membranes. Therefore, these films have similar electrical properties, but very different acid/base properties. The treated enzyme immobilization membranes maintain their neutral pH over a wide range of buffer pHs. In light of these advantages, the preferred enzyme immobilization material is a quaternary ammonium salt treated perfluoro sulfonic acid-PTFE copolymer (or modified perfluorinated ion exchange polymer)(modified Nafion® or modified Flemion®) membrane. More preferably, the enzyme immobilization material is a TBAB-modified Nafion® membrane material. Even more preferably, the enzyme immobilization material is a triethylhexylammonium bromide-modified Nafion® membrane material, phenyltrimethylammonium bromide-modified Nafion® membrane material, or a trimethyloctylammonium bromide-modified Nafion® membrane material.

f. Biocathode Embodiments

[0085] Advantageously, in many of the various embodiments, the concentration of the electrocatalyst or electron mediator in the enzyme immobilization material is sufficient to make the enzyme immobilization material conduct electrons. The concentration of the electrocatalyst or electron mediator in the enzyme immobilization material is from about 10 mM to about 3 M, more preferably from about 250 mM to about 2.25 M, still more preferably from about 500 mM to about 2 M, and most preferably from about 1.0 M to about 1.5 M. This concentration of electrocatalyst or electron mediator facilitates a rate of electron transfer that allows for maximization of the current density. When the enzyme immobilization material is a polymer, preferably, the above concentrations of the electrocatalyst or electron mediator alter the electronic properties of the polymeric enzyme immobilization material to make it a redox polymer.

[0086] Various biocathodes can be incorporated into the biofuel cells of the present invention. For example, biocathodes described in U.S. Patent Application No. 10/931,147 (published as U.S. Patent Application Publication No. 2005/0095466), herein incorporated by reference in its entirety.

2. Bioanode

[0087] In one embodiment, the bioanode comprises an electron conductor and an enzyme which is immobilized in an enzyme immobilization material wherein the macroscopic shape of the bioanode is non-planar and the non-planar bioanode has an increased surface area relative to a macroscopically planar bioanode. In another embodiment, the bioanode optionally further comprises an electrocatalyst for an electron mediator. An electrocatalyst can be absent from the bioanode when the bioanode contacts an electron mediator that is capable of undergoing a reversible redox reaction at the electron conductor. The above-identified components of the bioanode are adjacent to one another; meaning they are physically or chemically connected by appropriate means. Other embodiments are detailed infra at I.A.1.f. As the components are generally the same as the biocathode components, the following discussion concerns the differences in composition of the respective elements and differences in function, where appropriate.

a. Electron Conductor

[0088] As with the biocathode, the bioanode's electron conductor can be organic or inorganic in nature as long as it is able to conduct electrons through the material. In one embodiment, the bioanode electron conductor is carbon cloth.

b. Electron Mediators

[0089] The bioanode electron mediator serves to accept or donate electron(s), readily changing from oxidized to reduced forms. The electron mediator is a compound that can diffuse into the immobilization material and/or be incorporated into the immobilization material. As with the biocathode, it is preferred that the electron mediator's diffusion coefficient is maximized.

[0090] Exemplary electron mediators are nicotinamide adenine dinucleotide (NAD^+), flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide phosphate (NADP), or pyrroloquinoline quinone (PQQ), or equivalents of each. Other exemplary electron mediators are phenazine methosulfate, dichlorophenol indophenol, short chain ubiquinones, potassium ferricyanide, a protein, a metalloprotein, and stellacyanin. In one preferred embodiment, the electron mediator at the bioanode is NAD^+.

[0091] Where the electron mediator cannot undergo a redox reaction at the electron conductor by itself, the bioanode comprises an electrocatalyst for an electron mediator which facilitates the release of electrons at the electron conductor. Alternatively, a reversible redox couple that has a standard reduction potential of $0.0V \pm 0.5$ V is used as the electron mediator. Stated another way, an electron mediator that provides reversible electrochemistry on the electron conductor surface can be used. The electron mediator is coupled with a naturally occurring enzyme that is dependent on that electron mediator, an enzyme modified to be dependent on that electron mediator, or a synthetic enzyme that is dependent on that electron mediator. Examples of electron mediators that provide reversible electrochemistry on the electron conductor surface is pyrroloquinoline quinone (PQQ), phenazine methosulfate, dichlorophenol indophenol, short chain ubiquinones and potassium ferricyanide. In this embodiment, the preferred electron mediator utilized with the bioanode is PQQ. Due to the capability of the electron mediator to provide reversible electrochemistry at the electron

conductor surface, no electrocatalyst is necessary to catalyze the redox reaction in this embodiment.

[0092] Preferred compounds that are substrates for electrocatalysis by the redox polymer of the bioanode include reduced adenine dinucleotides, such as NADH, $FADH_2$ and NADPH.

c. Electrocatalyst for an Electron Mediator

[0093] Generally, the electrocatalyst is a substance that facilitates the release of electrons at the electron conductor. Stated another way, the electrocatalyst improves the kinetics of a reduction or oxidation of an electron mediator so the electron mediator reduction or oxidation can occur at a lower standard reduction potential. The electrocatalyst can be reversibly oxidized at the bioanode to produce electrons and thus, electricity. When the electrocatalyst is adjacent to the electron conductor, the electrocatalyst and electron conductor are in electrical contact with each other, but not necessarily in physical contact with each other. In one embodiment, the electron conductor is part of, associates with, or is adjacent to an electrocatalyst for an electron mediator.

[0094] Generally, the electrocatalyst can be an azine, a conducting polymer or an electroactive polymer. Exemplary electrocatalysts are methylene green, methylene blue, luminol, nitro-fluorenone derivatives, azines, osmium phenanthrolinedione, catechol-pendant terpyridine, toluene blue, cresyl blue, nile blue, neutral red, phenazine derivatives, tionin, azure A, azure B, toluidine blue O, acetophenone, metallophthalocyanines, nile blue A, modified transition metal ligands, 1,10-phenanthroline-5,6-dione, 1,10-phenanthroline-5,6-diol, $[Re(phen\text{-}dione)(CO)_3Cl]$, $[Re(phen\text{-}dione)_3](PF_6)_2$, poly(metallophthalocyanine), poly(thionine), quinones, diimines, diaminobenzenes, diaminopyridines, phenothiazine, phenoxazine, toluidine blue, brilliant cresyl blue, 3,4-dihydroxybenzaldehyde, poly(acrylic acid), poly(azure I), poly(nile blue A), poly(methylene green), poly(methylene blue), polyaniline, polypyridine, polypyrole, polythiophene, poly(thieno[3,4-b]thiophene), poly(3-hexylthiophene), poly(3,4-ethylenedioxypyrrole), poly(isothianaphthene), poly(3,4-ethylenedioxythiophene), poly(difluoroacetylene), poly(4-dicyanomethylene-4H-cyclopenta[2,1-*b*;3,4-*b'*]dithiophene), poly(3-(4-fluorophenyl)thiophene), poly(neutral red), a protein, a metalloprotein, or stellacyanin. In one preferred embodiment, the electrocatalyst for the electron mediator is poly(methylene green).

d. Enzyme

[0095] An enzyme catalyzes the oxidation of the fuel fluid at the bioanode. As enzymes also reduce an oxidant at the biocathode, they are more generally described above at I.A.1.d. Generally, naturally-occurring enzymes, man-made enzymes, artificial enzymes and modified naturally-occurring enzymes can be utilized. In addition, engineered enzymes that have been engineered by natural or directed evolution can be used. Stated another way, an organic or inorganic molecule that mimics an enzyme's properties can be used in an embodiment of the present invention.

[0096] Specifically, exemplary enzymes for use in a bioanode are oxidoreductases. In one preferred embodiment, the oxidoreductases act on the CH-OH group or CH-NH group of the fuel (alcohols, ammonia compounds, carbohydrates, aldehydes, ketones, hydrocarbons, fatty acids and the like).

[0097] In another preferred embodiment, the enzyme is a dehydrogenase. Exemplary enzymes in this embodiment include alcohol dehydrogenase, aldehyde dehydrogenase, formate dehydrogenase, formaldehyde dehydrogenase, glucose dehydrogenase, glucose oxidase, lactatic dehydrogenase, lactose dehydrogenase or pyruvate dehydrogenase. Preferably, the enzyme is an alcohol dehydrogenase (ADH).

[0098] In a presently preferred embodiment, the enzyme is a PQQ-dependent alcohol dehydrogenase. PQQ is the coenzyme of PQQ-dependent ADH and remains electrostatically attached to PQQ-dependent ADH and therefore the enzyme will remain in the membrane leading to an increased lifetime and activity for the biofuel cell. The PQQ-dependent alcohol dehydrogenase enzyme is extracted from gluconobacter. When extracting the PQQ-dependent ADH, it can be in two forms: (1) the PQQ is electrostatically bound to the PQQ-dependent ADH or (2) the PQQ is not electrostatically bound the PQQ-dependent ADH. For the second form where the PQQ is not electrostatically bound to the PQQ-dependent ADH, PQQ is added to the ADH upon assembly of the bioanode. In a presently preferred embodiment, the PQQ-dependent ADH is extracted from gluconobacter with the PQQ electrostatically bound.

e. Enzyme Immobilization Material

[0099] As noted above at I.A.1 and I.A.2., an enzyme immobilization material is utilized in the biofuel cell at the bioanode and/or the biocathode. Further detail regarding the

composition of the enzyme immobilization material and the immobilization mechanism can be found supra at I.A.1.e. In one embodiment, the bioanode's enzyme immobilization material is permeable to the fuel fluid and immobilizes and stabilizes the enzyme. The immobilization material is permeable to the fuel fluid so the oxidation of the fuel fluid at the bioanode can be catalyzed by the immobilized enzyme. Preferably, the enzyme immobilization material is a quaternary ammonium salt treated perfluoro sulfonic acid-PTFE copolymer (or modified perfluorinated ion exchange polymer)(modified Nafion® or modified Flemion®) membrane. More preferably, the enzyme immobilization material is a tetrabutylammonium bromide (TBAB) treated Nafion® membrane material. Even more preferably, the enzyme immobilization material is a triethylhexylammonium bromide treated Nafion® membrane material, a trimethyloctylammonium bromide treated Nafion® membrane material, or a phenyltrimethylammonium bromide treated Nafion® membrane material.

f. Bioanode Embodiments

[00100] Various preferred bioanodes are described in U.S. patent application 10/617,452 (published as U.S. Patent Application Publication No. 2004/0101741), which is incorporated herein by reference in its entirety.

B. Fuel Fluid and Oxidant

[00101] A fuel fluid that can be oxidized to produce electrons at the bioanode and an oxidant that can be reduced to produce water at the biocathode are components of the biofuel cell of this invention.

[00102] The fuel fluid for the bioanode is consumed in the oxidation reaction of the electron mediator and the immobilized enzyme. The fuel fluid's molecular size is small enough so the diffusion coefficient through the enzyme immobilization material is large. Exemplary fuel fluids are hydrogen, ammonia, alcohols (such as methanol, ethanol, propanol, isobutanol, butanol and isopropanol), allyl alcohols, aryl alcohols, glycerol, propanediol, mannitol, glucuronate, aldehyde, carbohydrates (such as glucose, glucose-1, D-glucose, L-glucose, glucose-6-phosphate, lactate, lactate-6-phosphate, D-lactate, L-lactate, fructose, galactose-1, galactose, aldose, sorbose and mannose), glycerate, coenzyme A, acetyl Co-A, malate, isocitrate, formaldehyde, acetaldehyde, acetate, citrate, L-gluconate, beta-

hydroxysteroid, alpha-hydroxysteroid, lactaldehyde, testosterone, gluconate, fatty acids, lipids, phosphoglycerate, retinal, estradiol, cyclopentanol, hexadecanol, long-chain alcohols, coniferyl-alcohol, cinnamyl-alcohol, formate, long-chain aldehydes, pyruvate, butanal, acyl-CoA, steroids, amino acids, flavin, NADH, $NADH_2$, NADPH, $NADPH_2$, hydrocarbons, and amines. In a preferred embodiment, the fuel fluid is an alcohol, more preferably methanol and/or ethanol; and most preferably ethanol.

[00103] The oxidant for the biocathode is consumed in the reduction reaction of the electron mediator and the immobilized enzyme using electrons supplied by the bioanode. The oxidant's molecular size is small enough so the diffusion coefficient through the enzyme immobilization material is large. A variety of means of supplying a source of the oxidant known in the art can be utilized.

[00104] In a preferred embodiment, the oxidant is gaseous oxygen, which is transported to the biocathode via diffusion. In another preferred embodiment, the oxidant is a peroxide compound.

II. Methods of Generating Electricity

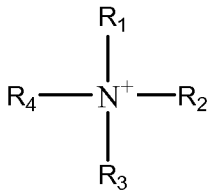
Among the various aspects of the present invention is a method of generating electricity using the biofuel cells as described above comprising (a) oxidizing the fuel fluid at the bioanode and reducing the oxidant at the biocathode; (b) oxidizing the reduced form of the electron mediator during the reduction of the oxidant at the biocathode; (c) oxidizing the electrocatalyst; and (d) reducing the electrocatalyst at the electron conductor.

Another aspect is a method of generating electricity using the biofuel cells described above comprising (a) oxidizing the fuel fluid at the bioanode and reducing the oxidant at the biocathode; (b) oxidizing the reduced form of the electron mediator during the reduction of the oxidant at the biocathode; and (c) reducing the electron mediator at the electron conductor.

Definitions

[00105] As used herein, the term "quaternary ammonium" or "quaternary ammonium salt" refers to a compound comprising nitrogen covalently bound to four organic groups, as illustrated in (2). N is nitrogen, R_1-R_4 are organic groups. Preferably, R_1, R_2, R_3 and R_4 are selected from the group consisting of propyl, butyl, pentyl or the like. In various

embodiments, preferably, R_1, R_2, R_3 and R_4 are the same organic group. In an alternate embodiment, R_1, R_2, and R_3 are a methyl or an ethyl group and R_4 is a hexyl, heptyl, octyl, nonyl, or decyl group. In yet another alternate embodiment, a quaternary phosphonium salt may be used, wherein the salt may be a quaternary phosphonium, such that the N^+ of Eq. 2 is replaced with a phosphorus ion. The counter ion to the quaternary ammonium (or phosphonium) ion may be any anion, such as for example a bromide ion (Br^-).



(2)

[00106] As used herein, a "fuel cell" comprises an anode and a cathode, which are separated to avoid an electrical short. A biofuel cell utilizes a fuel fluid and an enzyme which catalyzes an oxidation of the fuel fluid. In one embodiment, a "biofuel cell" utilizes organic fuels as a source of energy and redox enzymes to catalyze the oxidation of the organic fuel. The terms "fuel cell" and "biofuel cell" are used interchangeably in throughout the instant disclosure. In one embodiment, the fuel cell of the instant invention may be used in applications that require an electrical supply, such as, but not limited to electronic devices and equipment, toys, internal medical devices, and electrically powered vehicles. In another embodiment, the fuel cell of the instant invention may be implanted into a living organism, wherein the organic fuel is derived from the organism and the fuel cell powers a device implanted in the living organism.

[00107] As used herein, the term "bioanode" is an anode comprising an enzyme that catalyzes the oxidation of a fuel fluid. In one embodiment, the term "bioanode" means an anode, which comprises a redox enzyme that catalyzes the oxidation of an organic fuel. An anode provides a source of electrons for an electrical circuit or electrical potential. As used herein, the term "biocathode" means a cathode, which comprises a redox enzyme that catalyzes the reduction of an oxidant.

[00108] The terms "hydrocarbon" and "hydrocarbyl" as used herein describe organic compounds or radicals consisting exclusively of the elements carbon and hydrogen. These moieties include alkyl, alkenyl, alkynyl, and aryl moieties. These moieties also include alkyl, alkenyl, alkynyl, and aryl moieties substituted with other aliphatic or cyclic hydrocarbon

groups, such as alkaryl, alkenaryl and alkynaryl. Unless otherwise indicated, these moieties preferably comprise 1 to 20 carbon atoms.

[00109] The "substituted hydrocarbyl" moieties described herein are hydrocarbyl moieties which are substituted with at least one atom other than carbon, including moieties in which a carbon chain atom is substituted with a hetero atom such as nitrogen, oxygen, silicon, phosphorous, boron, sulfur, or a halogen atom. These substituents include halogen, heterocyclo, alkoxy, alkenoxy, alkynoxy, aryloxy, hydroxy, protected hydroxy, keto, acyl, acyloxy, nitro, amino, amido, nitro, cyano, thiol, ketals, acetals, esters and ethers.

[00110] Unless otherwise indicated, the alkyl groups described herein are preferably lower alkyl containing from one to eight carbon atoms in the principal chain and up to 20 carbon atoms. They may be straight or branched chain or cyclic and include methyl, ethyl, propyl, isopropyl, butyl, hexyl and the like.

[00111] Unless otherwise indicated, the alkenyl groups described herein are preferably lower alkenyl containing from two to eight carbon atoms in the principal chain and up to 20 carbon atoms. They may be straight or branched chain or cyclic and include ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, hexenyl, and the like.

[00112] Unless otherwise indicated, the alkynyl groups described herein are preferably lower alkynyl containing from two to eight carbon atoms in the principal chain and up to 20 carbon atoms. They may be straight or branched chain and include ethynyl, propynyl, butynyl, isobutynyl, hexynyl, and the like.

[00113] The terms "aryl" or "ar" as used herein alone or as part of another group denote optionally substituted homocyclic aromatic groups, preferably monocyclic or bicyclic groups containing from 6 to 12 carbons in the ring portion, such as phenyl, biphenyl, naphthyl, substituted phenyl, substituted biphenyl or substituted naphthyl. Phenyl and substituted phenyl are the more preferred aryl.

[00114] The terms "halogen" or "halo" as used herein alone or as part of another group refer to chlorine, bromine, fluorine, and iodine.

[00115] The term "acyl," as used herein alone or as part of another group, denotes the moiety formed by removal of the hydroxyl group from the group --COOH of an organic carboxylic acid, e.g., RC(O)-, wherein R is R_1, R_1O-, R_1R_2N-, or R_1S-, R_1 is hydrocarbyl,

heterosubstituted hydrocarbyl, or heterocyclo, and R_2 is hydrogen, hydrocarbyl or substituted hydrocarbyl.

[00116] The term "acyloxy," as used herein alone or as part of another group, denotes an acyl group as described above bonded through an oxygen linkage (--O--), e.g., RC(O)O- wherein R is as defined in connection with the term "acyl."

[00117] The term "heteroatom" shall mean atoms other than carbon and hydrogen.

The terms "heterocyclo" or "heterocyclic" as used herein alone or as part of another group denote optionally substituted, fully saturated or unsaturated, monocyclic or bicyclic, aromatic or nonaromatic groups having at least one heteroatom in at least one ring, and preferably 5 or 6 atoms in each ring. The heterocyclo group preferably has 1 or 2 oxygen atoms, 1 or 2 sulfur atoms, and/or 1 to 4 nitrogen atoms in the ring, and may be bonded to the remainder of the molecule through a carbon or heteroatom. Exemplary heterocyclo include heteroaromatics such as furyl, thienyl, pyridyl, oxazolyl, pyrrolyl, indolyl, quinolinyl, or isoquinolinyl and the like. Exemplary substituents include one or more of the following groups: hydrocarbyl, substituted hydrocarbyl, keto, hydroxy, protected hydroxy, acyl, acyloxy, alkoxy, alkenoxy, alkynoxy, aryloxy, halogen, amido, amino, nitro, cyano, thiol, ketals, acetals, esters and ethers.

[00118] The terms "hydroxyl protecting group" and "hydroxy protecting group" as used herein denote a group capable of protecting a free hydroxyl group ("protected hydroxyl") which, subsequent to the reaction for which protection is employed, may be removed without disturbing the remainder of the molecule. A variety of protecting groups for the hydroxyl group and the synthesis thereof may be found in "Protective Groups in Organic Synthesis" by T. W. Greene, John Wiley and Sons, 1981, or Fieser & Fieser. Exemplary hydroxyl protecting groups include methoxymethyl, 1-ethoxyethyl, benzyloxymethyl, (.beta.-trimethylsilylethoxy)methyl, tetrahydropyranyl, 2,2,2-trichloroethoxycarbonyl, t-butyl(diphenyl)silyl, trialkylsilyl, trichloromethoxycarbonyl and 2,2,2-trichloroethoxymethyl.

[00119] The following examples illustrate the invention.

EXAMPLES

EXAMPLE 1: Bioelectrode Assemblies

[00120] A bioanode prepared as described in U.S. Patent Application Serial No. 10/617,452 (published as U.S. Patent Application Publication No. 2004/0101741) and a biocathode prepared as described in U.S. Patent Application Serial No. 10/931,147 (published as U.S. Patent Application Publication No. 2005/0095466) are separated by a porous insulating material. The bioelectrode assembly having a bioanode and biocathode separated with a porous insulating material is then manipulated to form an alternating, bent, arcuate, folded, zigzag, spiral, coiled, or wound shape.

[00121] Another bioelectrode assembly is formed by preparing a bioanode as described in U.S. Patent Application Serial No. 10/617,452 (published as U.S. Patent Application Publication No. 2004/0101741) and preparing a biocathode as described in U.S. Patent Application Serial No. 10/931,147 (published as U.S. Patent Application Publication No. 2005/0095466). The bioanode is wrapped by a porous insulating material and then manipulated to form an alternating, bent, arcuate, folded, zigzag, spiral, coiled, or wound shape.

[00122] In another bioelectrode assembly is formed by preparing a bioanode as described in U.S. Patent Application Serial No. 10/617,452 (published as U.S. Patent Application Publication No. 2004/0101741) and preparing a biocathode as described in U.S. Patent Application Serial No. 10/931,147 (published as U.S. Patent Application Publication No. 2005/0095466). The biocathode is wrapped by a porous insulating material and then manipulated to form an alternating, bent, arcuate, folded, zigzag, spiral, coiled, or wound shape.

EXAMPLE 2: Calculation of Power Electronics

[00123] Various parameters can be calculated from the following formulae.

Electrode area (cm^2)	$A = 2\pi d \cdot \sum_{r=0}^{\left\lfloor \frac{d-t}{2t} \right\rfloor} (.025 + .05r)$ (3)
Power (W)	$P = A \cdot \sigma_P$ (4)
Maximum current (A)	$I_{max} = \frac{P}{v_{op}}$ (5)
Fuel volume (cm^3)	$V = \pi \left(\frac{d}{2}\right)^2 \cdot l \cdot \alpha$ (6)
Capacity (Wh)	$E = V \cdot \rho_E$ (7)
Charge (Ah)	$C = \frac{E}{v_{op}}$ (8)
Minimum running time (h)	$T = \frac{E}{I_{max} \cdot v_{op}}$ (9)

[00124] For formulae 3 to 9, r is the radius of the spiral in cm, d is the diameter of the spiral in cm, l is the length of the spiral in cm, t is the thickness of an electrode stack (assumed to be 0.05 cm as described below), v_{op} is the operating voltage and equals 1.5 V for alkaline batteries, α is the fuel to volume ratio, σ_p is the power density of the cell in W/cm^2 (assumed to be 0.010 W/cm^2 as described below), and ρ_E is the energy density in Wh/cm^3 (assumed to be 1.87 Wh/cm^3 as described below). Using the equations above and values for the variables, the following quantities are calculated: A (the electrode area in cm^2), P (the power in W), I_{max} (the maximum current in A), V (the fuel volume in cm^3), E (the capacity in Wh), C (the charge in Ah), and T (the running time in h).

[00125] The table below shows capacity and charge for fuel cells made in standard battery sizes as compared to that of standard alkaline cell batteries.

Size	**AAA**	**AA**	**C**	**D**
Diameter (cm)	1.05	1.42	2.6	3.3
Length (cm)	4.45	5	4.6	5.8
Electrode area (cm^2)	84.85	153.94	488.45	992.15
Cell power (W)	0.8485	1.5394	4.8845	9.9215
Maximum current @ 1.5V (A)	0.5657	1.0263	3.3564	6.6143
Fuel volume (cm^3)	1.926	3.959	12.211	24.804

Capacity (Wh)	3.6028	7.4037	22.835	46.383
Charge (Ah @1.5V)	2.4	4.9	15.2	30.9
Minimum running time (h)	4.2597	4.8095	4.675	4.675
Capacity of alkaline cell (Wh)	1.5	3.15	10.5	21
Charge of alkaline cell (Ah @ 1.5V)	1	2.1	7	14

EXAMPLE 3: Device Designs

[00126] Based upon the power electronic calculations from example 1, a cellular telephone booster power source is depicted in Figure 2. The power source is designed to maximize the talk-time and general battery life of the cellular telephone. The power source design (see Figure 2) packages three biofuel cells (green cylinders) with the same dimensions as standard AAA batteries. These cells are contained within a molded plastic piece that fits comfortably around this sample phone (slightly opaque figure on the back of the phone). The charger uses three spiral cells placed in a rectangular compartment. To provide room for extra fuel fluid, the spiral electrodes do not run the whole length of the compartment. In addition to the biofuel cells, drive electronics (turquoise shape near the bottom of the opaque figure) are contained within the plastic molding that are designed to increase the voltage of the biofuel cells to the desired operating voltage of the phone. These drive electronics along with the biofuel cells plug in to the phone's normal charging port. The device has the capability to both charge the battery and run the telephone itself for well over the normal limitations of the telephone's conventional battery.

[00127] In performing the calculations below, 100% efficiency of the power conversion electronics was assumed. From known talk-times and battery capacities for conventional cellular telephone batteries, it was estimated that the nominal power requirements of a cellular phone are a talk current (I_{talk}) of 350 mA, a talk voltage (v_{talk}) of 3.6V, a talk+charge current ($I_{talk+charge}$) of 350 mA, a talk+charge voltage ($v_{talk+charge}$) of 6.0 V, and a charge time while talking (T_{charge}) of 3 h. Using the cell configuration above wherein a rectangular compartment houses three spiral cells, the total electrode area, fuel volume, and cell power capabilities were calculated as follows.

Electrode area:

diameter (d) = 1.05 cm

length (l) = 3.68 cm

Electrode area (A_{spiral}) = 69.96 cm^2

Total electrode area (A) = $3 \cdot A_{spiral} = 209.8\ cm^2$

Fuel volume = Compartment volume – Electrode volume

Compartment dimensions: 1.05 cm x 4.45 cm x 4 cm

$$= 1.05 \cdot 4.45 \cdot 4 - \pi \left(\frac{1.05}{2}\right)^2 \cdot 3.68 \frac{cm}{spiral} \cdot 3spirals \cdot 0.5$$

$$= 18.69 - 1.593$$

$$= 17.097\ mL$$

Cell Power Capabilities

Cell power (P) = $209.8 \cdot 0.010 = 2.098$ W

Maximum charging current ($I_{max,\ 6.0V}$) = $\frac{2.098}{6.0} = 0.350A$

Maximum talk current ($I_{max,\ 3.6V}$) = $\frac{2.098}{3.6} = 0.583A$

Capacity (E) = $17.098 \cdot 1.87 = 31.97$ Wh

Talk time (T_{talk}) = $\frac{31.97}{3.6 \cdot 0.350} = 25.37$ h

Charge time ($T_{charge+talk}$) = $\frac{31.97}{6.0 \cdot 0.350} = 15.22$ h

[00128] Assuming that charging a conventional battery used in a cellular telephone takes about three hours at 350mA and 6.0V, the total energy used while charging is $3 \cdot 6.0 \cdot 0.350 = 6.3$ Wh. So, after charging (while talking), the cell still has 25.67 Wh of energy left (31.97 Wh – 6.3 Wh). The remaining talk time is 20.37 h ($25.67/(3.6 \cdot 0.350)$). The total talk time, from dead battery and full fuel cell to dead fuel cell and full battery is 23.37 hours.

[00129] Unless the battery is completely discharged, it will be able to absorb any current spikes or large transients. Also, for each additional mL of fuel fluid in the compartment, 1.48 hours ($25.67/(3.6 \cdot 0.350)$ of talk time is gained. If the compartment is relatively large as compared to the spirals, the fuel will be mixed during phone usage (e.g., by motion of the phone during talk time) to keep unreacted fuel in contact with the electrode assembly to maximize power. In addition, to improve the fuel-electrode distribution, the spiral electrode assemblies can be loosened inside the compartment.

[00130] Based upon the power electronic calculations from example 1, a laptop booster power source is depicted in Figure 3. This device consists of a molded plastic shape comparable if not identical in size to many conventional laptop batteries currently available on the consumer market. Eight fuel cells (Red cylinders), each approximately the size of a standard "C" size battery are contained within one side of the molded plastic (opaque figure). On the other side there is a small lithium ion battery (gray cylinder) and a set of drive electronics (green block). The drive electronics serve the same purpose as above for the cellular telephone booster device. The lithium ion battery serves as a supplement to the fuel cell, absorbing the current spikes and start-up transients inherent in any electronic device. This power source also has the capability to run the laptop for a much longer period of time than the conventional battery.

[00131] From device run times and battery sizes, the power requirements of a Dell™ Inspiron PP07S are estimated as a run current (I_{run}) of 1.26 A, a run voltage (v_{run}) of 14.8 V and a charge voltage (v_{charge}) of 19.5 V. From the cell configuration (see Figure 3), the "C" cell parameters are a diameter of 1.7 cm and a length of 26.5 cm and the lithium battery capacity of 1100 mAh. These parameters provide combined cell capabilities as follows.

$$I_{\max,14.8V} = 813mA$$
$$I_{\max,19.5V} = 617mA$$
$$T_{run} = \frac{1.1Ah}{V_{run} \cdot (I_{run} - I_{\max,14.8V})} = 2.46h$$
$$T_{charge} = \frac{1.1Ah}{V_{charge} \cdot I_{\max,19.5V}} = 1.35h$$
$$T_{\text{cell life}} = 18.7h$$

Notably, $I_{\max,14.8V}$ is less than the required 1.26A and the lithium ion battery can supply the remaining 447mA. At this rate, the battery will last for T_{run} = 2 hours, 27 minutes. After the maximum run time is completed, the laptop will need to be shut off for T_{charge} to allow the fuel cell to recharge the battery. This process can be repeated for a total of 18.7 hours.

[00132] When the ratio between the biofuel cell size and the battery size is set to 2/3, it is possible to achieve a run time of approximately two hours with a charge time of only 17 minutes. When using this setup, the charge/run cycle can be repeated for almost 40 hours.

The relationship of the running time, charging time and cell life to the percent of the power source occupied by the biofuel cell is represented in the graphs in Figure 4.

[00133] In view of the above, it will be seen that the several objects of the invention are achieved and other advantageous results attained.

[00134] As various changes could be made in the above methods without departing from the scope of the invention, it is intended that all matter contained in the above description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

[00135] Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims, which follow the examples.

What is claimed is:

1. A bioanode comprising

(a) an electron conductor;

(b) at least one anode enzyme capable of reacting with an oxidized form of the electron mediator and a fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator, the reduced form of the electron mediator being capable of releasing electrons to the electron conductor; and

(c) an enzyme immobilization material being permeable to the fuel fluid and the electron mediator;

wherein the macroscopic shape of the bioanode is non-planar and the surface area of the bioanode is greater than that of an otherwise identical macroscopically planar bioanode.

2. A bioanode comprising

(a) an electron conductor;

(b) at least one anode enzyme capable of reacting with an oxidized form of an electron mediator and a fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator, the reduced form of the electron mediator being capable of releasing electrons to the electron conductor; and

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being permeable to the fuel fluid;

wherein the macroscopic shape of the bioanode is non-planar and the surface area of the bioanode is greater than that of an otherwise identical macroscopically planar bioanode.

3. A bioanode comprising

(a) an electron conductor;

(b) at least one anode enzyme capable of reacting with an oxidized form of the electron mediator and a fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(c) an enzyme immobilization material being permeable to the fuel fluid and the electron mediator; and

(d) an electrocatalyst adjacent the electron conductor, an oxidized form of the electrocatalyst being capable of reacting with the reduced form of the electron mediator to produce an oxidized form of the electron mediator and a reduced form of the electrocatalyst,

the reduced form of the electrocatalyst being capable of releasing electrons to the electron conductor;

wherein the macroscopic shape of the bioanode is non-planar and the surface area of the bioanode is greater than that of an otherwise identical macroscopically planar bioanode.

4. A bioanode comprising

(a) an electron conductor;

(b) at least one anode enzyme capable of reacting with an oxidized form of an electron mediator and a fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being permeable to the fuel fluid; and

(d) an electrocatalyst adjacent the electron conductor, an oxidized form of the electrocatalyst being capable of reacting with the reduced form of the electron mediator to produce an oxidized form of the electron mediator and a reduced form of the electrocatalyst, the reduced form of the electrocatalyst being capable of releasing electrons to the electron conductor;

wherein the macroscopic shape of the bioanode is non-planar and the surface area of the bioanode is greater than that of an otherwise identical macroscopically planar bioanode.

5. A biocathode comprising:

(a) an electron conductor;

(b) at least one cathode enzyme capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and

(c) an enzyme immobilization material comprising an electrocatalyst, the enzyme immobilization material being permeable to the oxidant, an oxidized form of the electrocatalyst being capable of gaining electrons from the electron conductor to produce a reduced form of the electrocatalyst that is capable of reacting with an oxidized form of the electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst; and

wherein the macroscopic shape of the biocathode is non-planar and the surface area of the biocathode is greater than that of an otherwise identical macroscopically planar biocathode.

6. A biocathode comprising:

(a) an electron conductor;

(b) at least one cathode enzyme capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being permeable to the oxidant, an oxidized form of the electron mediator being capable of gaining electrons from the electron conductor to produce a reduced form of the electron mediator; and

wherein the macroscopic shape of the biocathode is non-planar and increases the surface area of the biocathode relative to a macroscopically planar biocathode.

7. A biocathode comprising:

(a) an electron conductor;

(b) at least one cathode enzyme capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and

(c) an enzyme immobilization material comprising the electron mediator and an electrocatalyst, the enzyme immobilization material being permeable to the oxidant, an oxidized form of the electrocatalyst being capable of gaining electrons from the electron conductor to produce a reduced form of the electrocatalyst that is capable of reacting with an oxidized form of the electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst; and

wherein the macroscopic shape of the biocathode is non-planar and the surface area of the biocathode is greater than that of an otherwise identical macroscopically planar biocathode.

8. A biocathode comprising:

(a) an electron conductor;

(b) at least one cathode enzyme capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being permeable to the oxidant, an oxidized form of an

electrocatalyst being capable of gaining electrons from the electron conductor to produce a reduced form of the electrocatalyst that is capable of reacting with an oxidized form of the electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst; and

wherein the macroscopic shape of the biocathode is non-planar and the surface area of the biocathode is greater than that of an otherwise identical macroscopically planar biocathode.

9. A biofuel cell for generating electricity comprising:
a fuel fluid;
an electron mediator;
a bioanode of any one of claims 1 to 4; and
a biocathode.

10. A biofuel cell for generating electricity comprising:
a fuel fluid;
an electron mediator;
a bioanode; and
a biocathode of any one of claims 5 to 8.

11. A biofuel cell for generating electricity comprising:
a fuel fluid;
an electron mediator;
a bioanode of any one of claims 1 to 4; and
a biocathode of any one of claims 5 to 8
wherein the bioanode and the biocathode are separated by a porous insulating material.

12. The biocathode, bioanode or biofuel cell of any one of claims 1 to 11 wherein the non-planar shape of the biocathode or bioanode is alternating, bent, arcuate, folded, zigzag, spiral, coiled, or wound.

13. The biocathode, bioanode or biofuel cell of any one of claims 1 to 12 wherein the non-planar macroscopic shape of the biocathode or bioanode has an electrode area at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more times greater than an otherwise identical biocathode or bioanode having a planar macroscopic shape.

14. The biofuel cell of any one of claims 11 to 13 wherein the porous insulating material is selected from a mesh fabricated from polypropylene, polyethylene, nylon, polyvinyl chloride, polytetrafluoroethylene, polyester, ethylene vinyl acetate, thermoplastic polymers, or styrenic block copolymers, and combinations thereof.

15. The biofuel cell of any one of claims 11 to 14 wherein the porous insulating material has a hole size from about 0.06 cm to about 0.75 cm.

16. The biofuel cell of any one of claims 11 to 15 wherein the porous insulating material has a thickness from about 0.1 cm to about 0.75 cm.

17. The biofuel cell of any one of claims 11 to 16 wherein the porous insulating material has a nominal open area from about 15% to about 70%.

18. The bioanode, biocathode or biofuel cell of any one of claims 1 to 17 wherein the enzyme immobilization material is capable of immobilizing and stabilizing the enzyme.

19. The biocathode of any one of claims 5 to 8 wherein the electron mediator is present in a concentration sufficient to make the enzyme immobilization material conduct electrons.

20. The bioanode, biocathode or biofuel cell of any one of claims 1 to 19 wherein the enzyme immobilization material comprises a micellar or inverted micellar structure.

21. The bioanode, biocathode or biofuel cell of any one of claims 1 to 19 wherein the enzyme immobilization material comprises a modified perfluoro sulfonic acid-PTFE copolymer.

22. The bioanode, biocathode or biofuel cell of any one of claims 1 to 21 wherein the electron conductor comprises a carbon-based material, a metallic conductor, a semiconductor, a metal oxide or a modified conductor.

23. The bioanode, biocathode or biofuel cell of any one of claims 1 to 21 wherein the electron conductor comprises a carbon-based material.

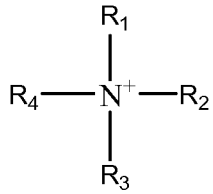
24. The bioanode, biocathode or biofuel cell of claim 23 wherein the electron conductor comprises carbon cloth, carbon paper, carbon screen printed electrodes, carbon black, carbon powder, carbon fiber, single-walled carbon nanotubes, double-walled carbon

nanotubes, multi-walled carbon nanotubes, carbon nanotube arrays, diamond-coated conductors, glass carbon, mesoporous carbon, graphite, uncompressed graphite worms, delaminated purified flake graphite, high performance graphite, highly ordered pyrolytic graphite, pyrolytic graphite or polycrystalline graphite.

25. The bioanode, biocathode or biofuel cell of any one of claims 1 to 24 wherein the enzyme immobilization material is modified with a hydrophobic cation larger than NH_4^+.

26. The bioanode, biocathode or biofuel cell of claim 25 wherein the hydrophobic cation comprises an ammonium-based cation, quaternary ammonium cation, alkyltrimethylammonium cation, organic cation, phosphonium cation, triphenylphosphonium, pyridinium cation, imidazolium cation, hexdecylpyridinium, ethidium, viologen, methyl viologen, benzyl viologen, bis(triphenylphosphine)iminium metal complex, bipyridyl metal complex, phenanthroline-based metal complex, $[Ru(bipyridine)_3]^{2+}$ or $[Fe(phenanthroline)_3]^{3+}$.

27. The bioanode, biocathode or biofuel cell of claim 25 wherein the hydrophobic cation comprises a quaternary ammonium cation represented by formula 2



(2)

wherein R_1, R_2, R_3 and R_4 are independently hydrogen, hydrocarbyl, substituted hydrocarbyl or heterocyclo wherein at least one of R_1, R_2, R_3 and R_4 is other than hydrogen.

28. The bioanode, biocathode or biofuel cell of claim 27 wherein R_1, R_2, R_3 and R_4 are independently hydrogen, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl or tetradecyl wherein at least one of R_1, R_2, R_3 and R_4 is other than hydrogen.

29. The bioanode, biocathode or biofuel cell of claim 27 wherein R_1, R_2, R_3 and R_4 are the same and are methyl, ethyl, propyl, butyl, pentyl or hexyl.

30. The bioanode, biocathode or biofuel cell of claim 21 wherein the modified perfluoro sulfonic acid-PTFE copolymer is modified with tetrabutylammonium halide, triethylhexylammonium halide or trimethyldodecylammonium halide.

31. The bioanode, biocathode or biofuel cell of claim 21 wherein the modified perfluoro sulfonic acid-PTFE copolymer is modified with tetrabutylammonium halide or triethylhexylammonium halide.

32. The bioanode, biocathode or biofuel cell of claim 21 wherein the modified perfluoro sulfonic acid-PTFE copolymer is modified with triethylhexylammonium halide.

33. The bioanode, biocathode or biofuel cell of any one of claims 1 to 32 wherein the enzyme comprises an oxidoreductase.

34. The bioanode or biofuel cell of any one of claims 1 to 4 and 9 to 33 wherein the anode enzyme comprises a glucose oxidase, alcohol-based oxidase or cholesterol-based oxidase.

35. The biocathode or biofuel cell of any one of claims 5 to 34 wherein the cathode enzyme comprises laccase, cytochrome C oxidase, bilirubin oxidase or peroxidase.

36. The biocathode or biofuel cell of any one of claims 1 to 4 and 9 to 34 wherein the anode enzyme comprises an oxygen oxidoreductase having an optimum activity at a pH between about 6.5 and about 7.5.

37. The biocathode or biofuel cell of any one of claims 5 to 33 wherein the cathode enzyme comprises bilirubin oxidase.

38. The biocathode or biofuel cell of any one of claims 5 to 37 wherein the electron mediator comprises a metalloprotein, a conjugated organic compound, a sugar, a sterol, a fatty acid or a coenzyme or substrate of an oxidase.

39. The biocathode or biofuel cell of any one of claims 5 to 37 wherein the oxidized form of the electron mediator comprises stellacyanin, bilirubin, glucose or cholesterol.

40. The biocathode or biofuel cell of any one of claims 5 to 37 wherein the oxidized form of the electron mediator comprises bilirubin.

41. The biocathode or biofuel cell of any one of claims 5 to 40 wherein the electrocatalyst for the electron mediator comprises organometallic cations with standard reduction potentials greater than +0.4 volts.

42. The biocathode or biofuel cell of any one of claims 5 to 40 wherein the electrocatalyst for the electron mediator comprises osmium, ruthenium, iron, nickel, rhodium, rhenium, or cobalt complexes.

43. The biocathode or biofuel cell of any one of claims 5 to 40 wherein the reduced form of the electrocatalyst for the electron mediator comprises $Ru(phen)_3^{+2}$, $Fe(phen)_3^{+2}$, $Ru(bpy)_3^{+2}$, $Os(bpy)_3^{+2}$ or $Os(terpy)_3^{+2}$.

44. The biocathode or biofuel cell of any one of claims 5 to 40 wherein the reduced form of the electrocatalyst for the electron mediator comprises $Ru(bpy)_3^{+2}$.

45. The biocathode or biofuel cell of any one of claims 5 to 44 wherein the concentration of the electrocatalyst is from about 10 mM to about 3 M.

46. The biocathode or biofuel cell of any one of claims 5 to 44 wherein the concentration of the electrocatalyst is from about 250 mM to about 2.25 M.

47. The biocathode or biofuel cell of any one of claims 5 to 44 wherein the concentration of the electrocatalyst is from about 500 mM to about 2 M.

48. The biocathode or biofuel cell of any one of claims 5 to 44 wherein the concentration of the electrocatalyst is from about 1 M to about 1.5 M.

49. The biofuel cell of any one of claims 9 to 48 wherein the oxidant comprises oxygen or peroxide.

50. The biofuel cell of any one of claims 9 to 48 wherein the oxidant comprises oxygen.

51. The biofuel cell of claim 50 wherein the oxidant comprises air.

52. The biofuel cell of any one of claims 9 to 51 wherein the fuel fluid comprises ammonia, methanol, ethanol, propanol, isobutanol, butanol and isopropanol, allyl alcohols, aryl

alcohols, glycerol, propanediol, mannitol, glucuronate, aldehyde, carbohydrates, glucose, glucose-1, D-glucose, L-glucose, glucose-6-phosphate, lactate, lactate-6-phosphate, D-lactate, L-lactate, fructose, galactose-1, galactose, aldose, sorbose, mannose, glycerate, coenzyme A, acetyl Co-A, malate, isocitrate, formaldehyde, acetaldehyde, acetate, citrate, L-gluconate, beta-hydroxysteroid, alpha-hydroxysteroid, lactaldehyde, testosterone, gluconate, fatty acids, lipids, phosphoglycerate, retinal, estradiol, cyclopentanol, hexadecanol, long-chain alcohols, coniferyl-alcohol, cinnamyl-alcohol, formate, long-chain aldehydes, pyruvate, butanal, acyl-CoA, steroids, amino acids, flavin, NADH, $NADH_2$, NADPH, $NADPH_2$ or hydrogen.

53. The biofuel cell of any one of claims 9 to 51 wherein the fuel fluid comprises methanol, ethanol or propanol.

54. The biofuel cell of any one of claims 9 to 51 wherein the fuel fluid comprises ethanol.

55. The biofuel cell of any one of claims 9 to 54 wherein the bioanode comprises a PQQ-dependent alcohol dehydrogenase.

56. The biofuel cell of any one of claims 9 to 54 wherein the bioanode comprises a PQQ-dependent alcohol dehydrogenase which has a PQQ molecule electrostatically associated with it.

57. The biofuel cell of any one of claims 9 to 55 wherein the bioanode and biocathode are not separated by a salt bridge or a polymer electrolyte membrane.

58. A method of generating electricity using the biofuel cell of any one of claims 9 to 57 comprising

(a) oxidizing the fuel fluid at the bioanode and reducing the oxidant at the biocathode;

(b) oxidizing the reduced form of the electron mediator during the reduction of the oxidant at the biocathode;

(c) oxidizing the electrocatalyst; and

(d) reducing the electrocatalyst at the electron conductor.

59. A method of generating electricity using the biofuel cell of any one of claims 9 to 57 comprising

(a) oxidizing the fuel fluid at the bioanode and reducing the oxidant at the biocathode;

(b) oxidizing the reduced form of the electron mediator during the reduction of the oxidant at the biocathode; and

(c) reducing the electron mediator at the electron conductor.

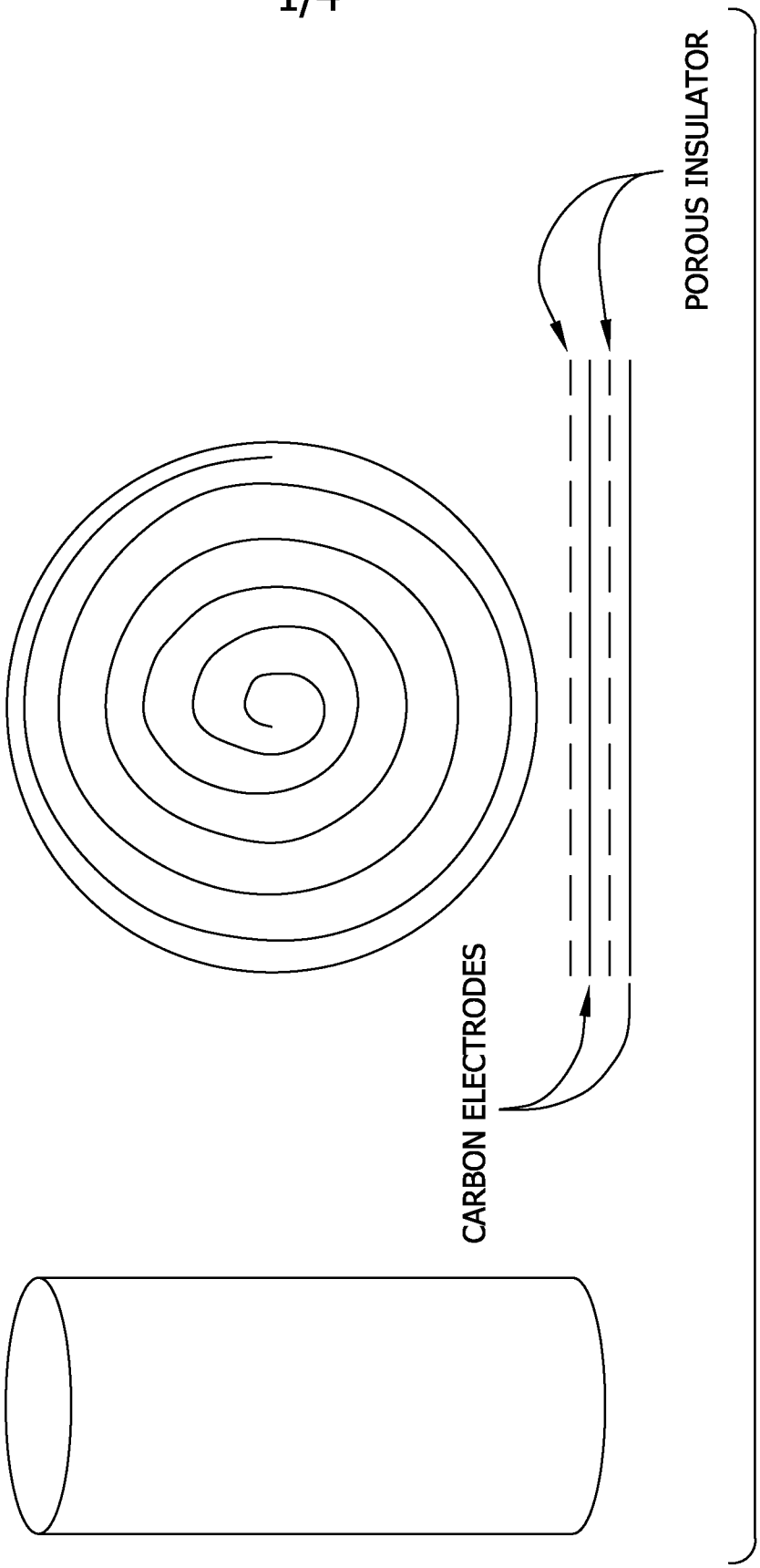


FIG. 1

FIG. 2

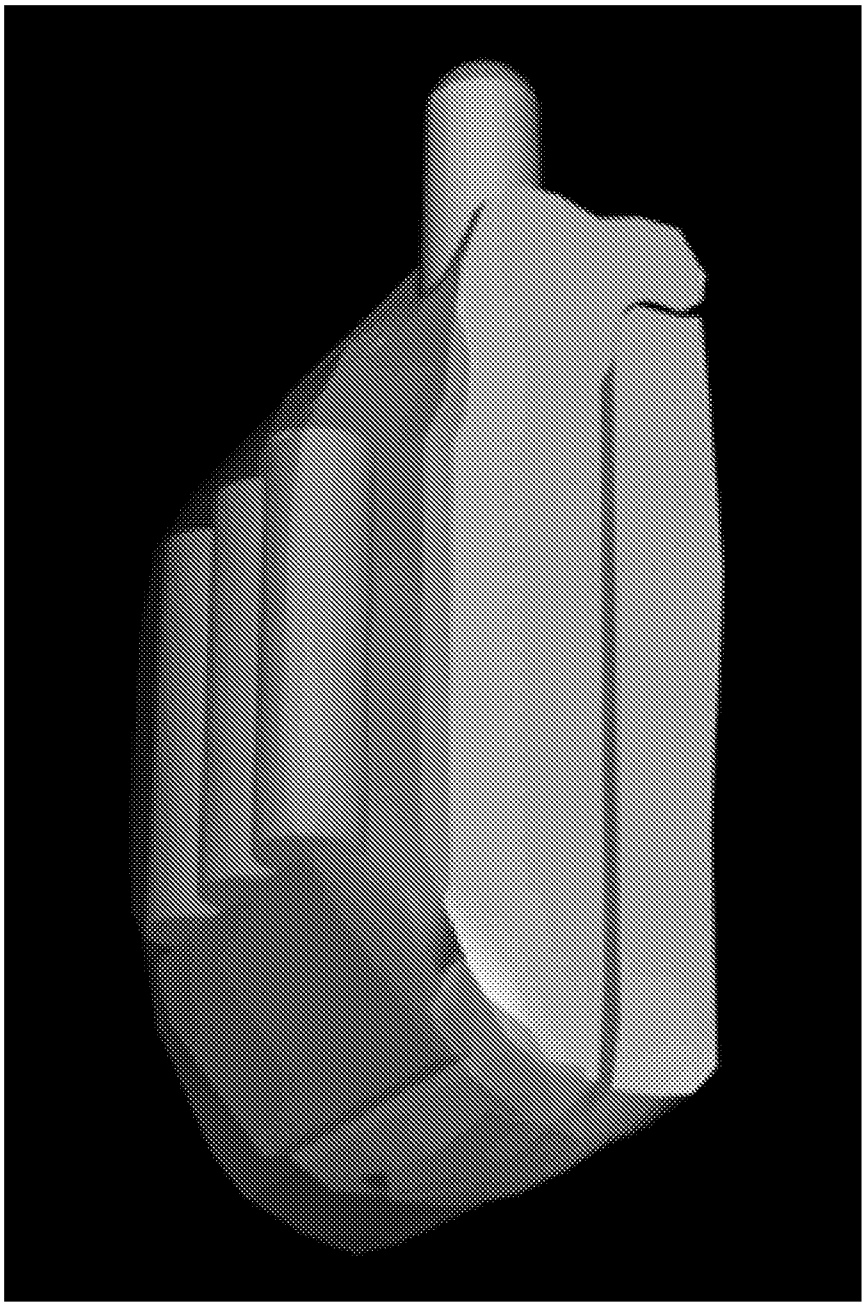
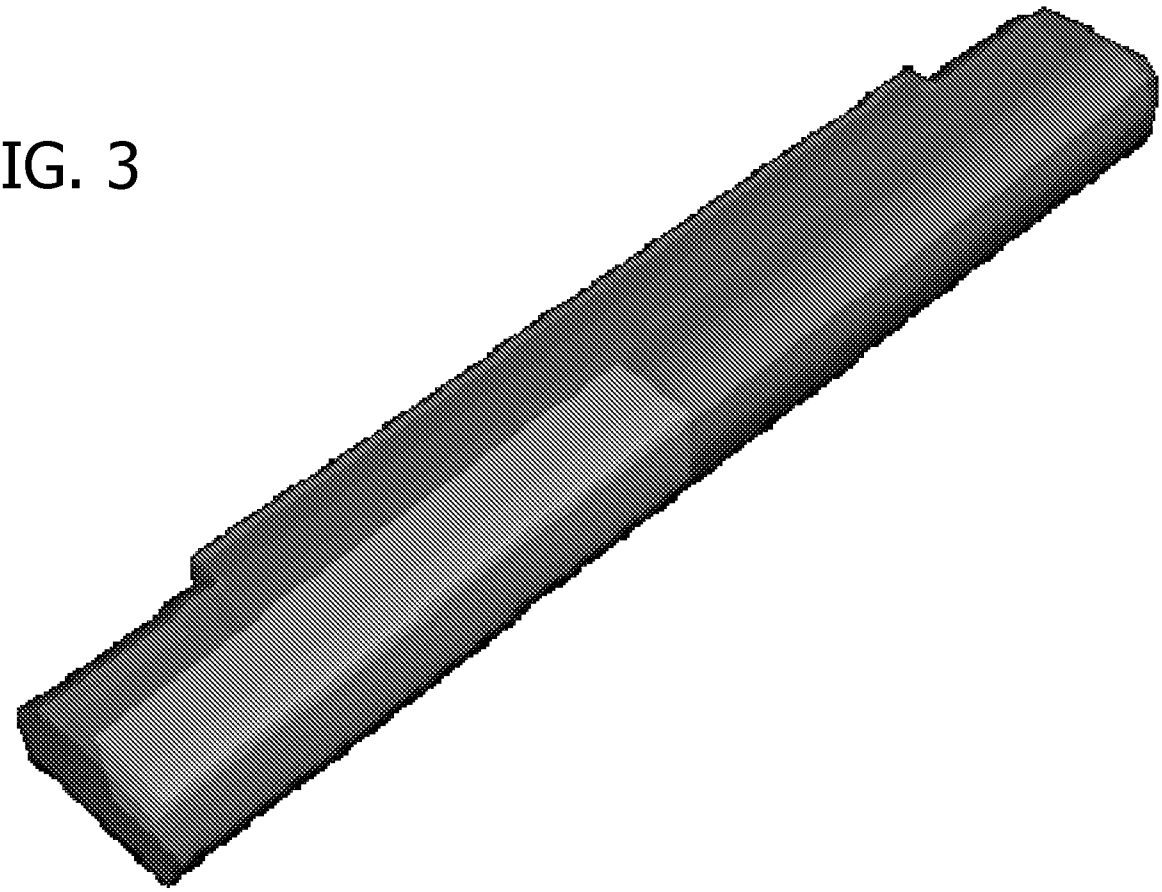


FIG. 3



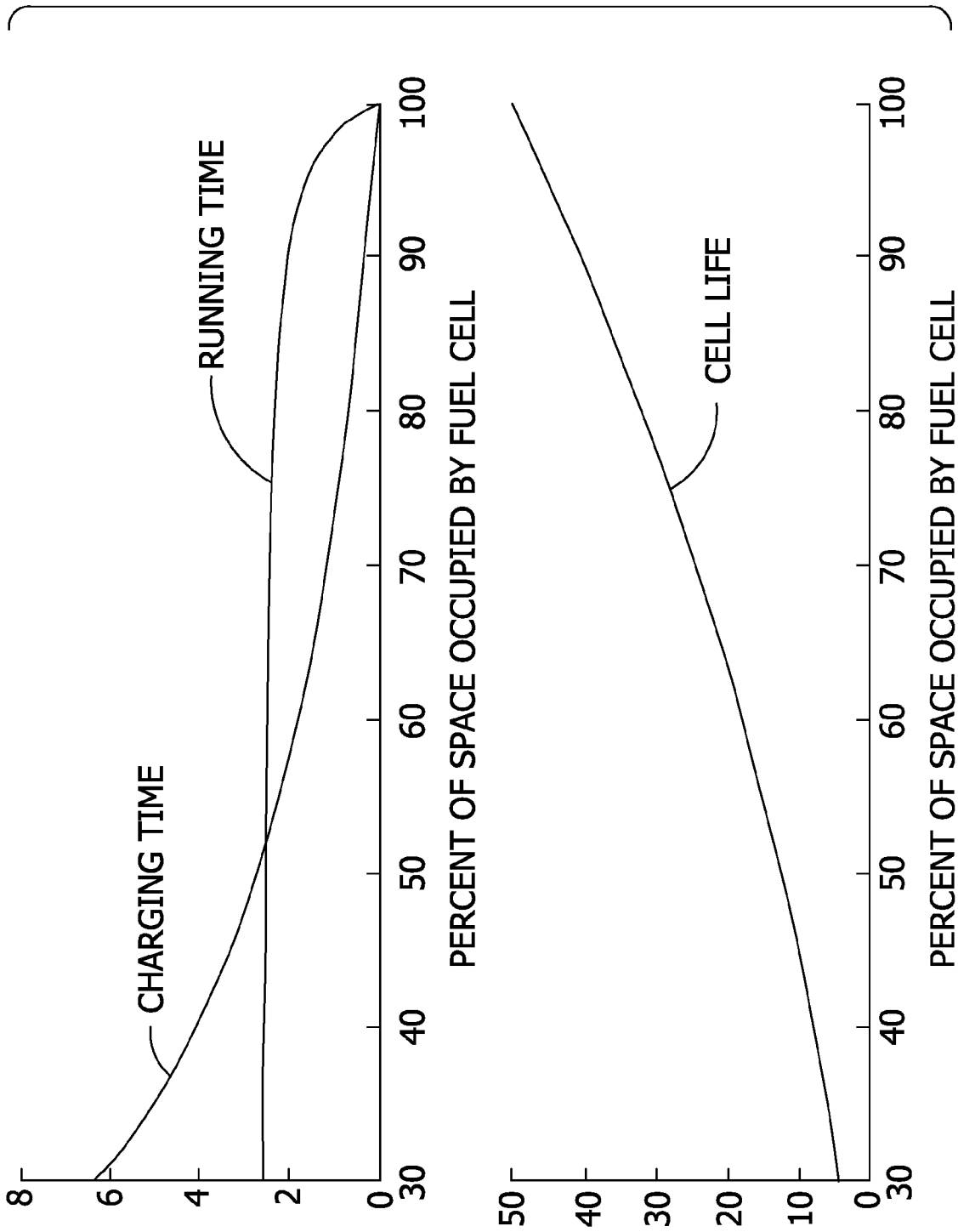

CHARGING TIME
RUNNING TIME
PERCENT OF SPACE OCCUPIED BY FUEL CELL
CELL LIFE
PERCENT OF SPACE OCCUPIED BY FUEL CELL

FIG. 4