

**A Porous Fiber, Methods of Making the Same and Uses
Thereof**

Technical Field

5 The present invention generally relates to a porous fiber. The present invention also relates to a method of making the porous fiber and uses thereof.

Background

10 Biological materials such as bacteria, virus, enzyme, algae, cell, yeast, protein or DNA have been utilized in a wide range of commercial applications. For example, biological material such as bacteria and yeast are used in water, air and soil treatments for the biodegradation and
15 removal of organic compounds and other contaminants such as metals and nitrates. However, the degradation of the contaminants in the environment depends on the type of biological material and its quantity present. When either of these is absent, the contaminant is not efficiently
20 degraded and removed from the environment. This is extremely disadvantageous, in particular, in the treatment of air and water pollution because speedy removal of the contaminants is desired in order to reduce the harmful impact of these contaminants on the environment.

25 Membrane processes are also widely used in removing contaminants from gas and liquid streams. Membranes are used to remove the contaminants by physical and chemical means. These membranes have been made from polymeric materials, inorganic materials or a combination of the
30 two. A combination of biological material and membranes in a reactor, or a membrane bioreactor, is a well known device that is utilized in a wide range of commercial applications.

However, a conventional bioreactor requires a start-up time for the specific biological material to grow before stable removal of the contaminant can be achieved. This start-up time can range from days to months for some
5 contaminant removal processes. Furthermore, if the specific biological material that carries out the degradation of the contaminant is absent, it can undermine the treatment process completely. Secondly, membrane fouling is a serious problem in all membrane processes and
10 this is also encountered in membrane bioreactors. Membrane fouling can significantly reduce the performance of the membrane process and increase energy and operating costs.

A biological material such as protein, nucleic acids, cell or enzyme can also be used as a biosensor in which
15 changes in an environmental system or sample can be detected by the biological material. A known biosensor comprises an encapsulated electrospun nanofiber. Electrospinning is a known technique for forming nanofibers. In order to exploit the properties of the
20 biological material, the biological material is encapsulated in a nanofiber. Encapsulation occurs when the biological material is electrospun together with the polymer forming the nanofiber. The formed nanofiber retains the biological material in the core of the
25 nanofiber and does not allow interaction between the biological material and an external environment.

Encapsulated nanofibers have not been used before in membrane filtration processes.

There is a need to create improved membranes with
30 biological materials encapsulated within, that overcomes, or at least ameliorates, one or more of the disadvantages described above.

Summary

The present disclosure provides a porous fiber having a core-shell configuration, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material.

In a first aspect, there is provided a porous fiber having a core-shell configuration, said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material therein.

The pores may be formed along the length of the fiber. Advantageously, the pores may allow the encapsulated biological material to be exposed to an external environment. The pores of the fiber may be dimensioned such that they do not allow the escape of the biological material from the fiber and at the same time, increase the contact between the biological material and the external environment.

In a conventional (non-porous) fiber, only the exposed ends of the fiber allow interaction of the encapsulated biological material with the external environment. However, in the disclosed porous fiber, the encapsulated biological material may interact with the external environment not only through the exposed ends, but also along the length of the fiber via the pores. Accordingly, the disclosed porous fiber increases the contact surface area between the encapsulated biological material and the environment. This increased contact area may aid in decreasing the reaction time when the encapsulated biological material acts on a target substrate in the external environment.

The present disclosure provides a method of forming a porous fiber having a core-shell configuration, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material, comprising the steps

5 of:

providing a core solution of the biological material;
providing a mixture comprising a fiber material and a pore-forming material as the shell solution, wherein the

fiber material and the pore forming material are miscible with each other;

forming a core-shell fiber from said core solution and shell solution, said fiber encapsulating and retaining
5 said biological material in the core; and
removing said pore-forming material from said formed core-shell fiber to create pores therein.

In a second aspect, there is provided a method of forming a porous fiber having a core-shell configuration,
10 said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material, comprising the steps of:

a. providing a core solution of the biological
15 material;

b. providing a mixture comprising a fiber material comprised of the non-biodegradable polymer and a pore-forming material as the shell solution, wherein the fiber material and the pore forming material are miscible with
20 each other;

c. forming a core-shell fiber from said core solution and shell solution, said fiber encapsulating and retaining said biological material in the core; and

d. removing said pore-forming material from said
25 formed core-shell fiber to create pores therein.

The present disclosure provides use of a porous fiber having a core-shell configuration, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material, in the removal of contaminants from
30 an environmental sample.

In a third aspect, there is provided the use of a porous fiber having a core-shell configuration, said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material therein, for the removal of contaminants from an environmental sample.

The present disclosure provides use of a porous fiber having a core-shell configuration, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material, as a biosensor.

In a fourth aspect, there is provided the use of a porous fiber having a core-shell configuration, said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material therein, as a biosensor.

The present disclosure provides a filtration membrane comprising a plurality of porous fibers, each porous fiber having a core-shell configuration, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material.

In a fifth aspect, there is provided a filtration membrane comprising a plurality of porous fibers, each porous fiber having a core-shell configuration, said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material therein.

The present disclosure provides a bioreactor comprising a membrane module, wherein said membrane module comprises a plurality of porous fibers, each porous fiber having a core-shell configuration, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material.

In a sixth aspect, there is provided a bioreactor comprising a membrane module, wherein said membrane module comprises a plurality of porous fibers, each porous fiber having a core-shell configuration, said fiber comprised of
5 a non-biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material therein.

The present disclosure provides a membrane contactor comprising a membrane module, wherein said membrane module
10 comprises a plurality of porous fibers, each porous fiber having a core-shell configuration, wherein the pores on the fiber are

configured to encapsulate and thereby retain a biological material.

In a seventh aspect, there is provided a membrane contactor comprising a membrane module, wherein said
5 membrane module comprises a plurality of porous fibers, each porous fiber having a core-shell configuration, said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material therein.

10

Definitions

The following words and terms used herein shall have the meaning indicated:

The term 'biological material' is to be interpreted
15 broadly to include any substance derived or obtained from a living organism. Examples of such biological material include, but are not limited to, bacteria, viruses, enzymes, algae, cells, yeast, proteins and nucleic acids.

The term "nano-sized", when referring to a nanofiber,
20 is to be interpreted broadly to relate to an average diameter of the nanofiber as being less than about 1000nm, less than about 500 nm, or less than about 100 nm. After encapsulating a biological material therein, the diameter of the nanofiber may be more than about 200 nm. When the
25 term "nano-sized" is used in relation to a particle size, this term relates to an average particle size of the particle of being less than about 1,000 nm. The particle size may refer to the diameter of the particles where they are substantially spherical. The particles may be non-
30 spherical and the particle size range may refer to the equivalent diameter of the particles relative to spherical particles.

The term "micro-sized", when referring to a microfiber, is to be interpreted broadly to relate to an average diameter of the microofiber as being more than about 1 μm , until about 10 μm . When the term "micro-
5 sized" is used in relation to a particle size, this term relates to an average particle size of the particle of being between about 1 μm to about 10 μm . The particle size may refer to the diameter of the particles where they are substantially spherical. The particles may be non-

spherical and the particle size range may refer to the equivalent diameter of the particles relative to spherical particles.

The term "nanofiber" is to be interpreted broadly to refer to a fiber which has a diameter in the nano-sized range.

The term "microfiber" is to be interpreted broadly to refer to a fiber which has a diameter in the micro-sized range.

The term "bioremediation" is to be interpreted broadly to refer to a managed or spontaneous process in which microbiological processes are used to degrade or transform contaminants to less toxic or non-toxic forms, thereby remedying, eliminating or removing environmental contamination. The term "bioremediation" is also applicable to the treatment of an environmental system or sample which contains contaminants that are not naturally present in that system or sample such as heavy metals or chlorinated compounds. Bioremediation may be carried out until the contaminant is no longer detectable in the contaminated system or sample or is reduced to an acceptable amount or concentration in the system or sample.

The term "contaminant", and grammatical variants thereof, is to be interpreted broadly to refer to any substance that imparts an undesirable, but not necessarily toxic, effect on the environment system or sample. For example, the contaminant may include, but is not limited to, organic materials such as aliphatic hydrocarbon compounds, aromatic-containing compounds and chlorinated compounds as well as inorganic materials such as metals and nitrates.

The word "substantially" does not exclude "completely" e.g. a composition which is "substantially

free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means $\pm 5\%$ of the stated value, more typically $\pm 4\%$ of the stated value, more typically $\pm 3\%$ of the stated value, more typically, $\pm 2\%$ of the stated value, even more typically $\pm 1\%$ of the stated value, and even more typically $\pm 0.5\%$ of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Certain embodiments may also be described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the

generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

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Detailed Disclosure of Embodiments

Exemplary, non-limiting embodiments of a porous fiber having a core-shell configuration, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material therein, a method of making the porous fiber and uses thereof will now be disclosed.

In the porous fiber, a plurality of pores may be present along the length of the fiber. The pores may be present on the surface of the fiber. The pores may extend into the fiber from the surface of the fiber such that a three-dimensional network of the pores may be envisaged.

The porosity of the fiber, which is defined as the ratio of the volume of the pores in the fiber to the total volume of the fiber, may be in the range selected from the group consisting of about 5% to about 90%, about 10% to about 90%, about 20% to about 90%, about 30% to about 90%, about 40% to about 90%, about 50% to about 90%, about 60% to about 90%, about 70% to about 90%, about 80% to about 90%, about 5% to about 80%, about 5% to about 70%, about 5% to about 60%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 5% to about 20%, about 5% to about 10% and about 30% to about 60%.

In one embodiment, the porous fiber has a generally longitudinal shape.

In one embodiment the biological material is dispersed throughout the length of the longitudinal fiber.

In another embodiment greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater than 90%, greater than 95%, greater than 99% of the porous fibers

comprise uniformly distributed pores along the length of the fiber. This is advantageous as the efficiency of the porous fiber is increased. For example in membrane applications, the high percentage of pores present in the fiber as disclosed herein results in an increase in the number of channels that can permit the interaction of the encapsulated biological material with the external environment of the fiber, whilst retaining the biological material within the core of the fiber. In one embodiment the percentage of fibers having pores is in the range of about 5% to 90%.

The average pore size of the pores may be in the range of about 1 nm to about 1000 nm, about 10 nm to about 1000 nm, about 100 nm to about 1000 nm, about 200 nm to about 1000 nm, about 300 nm to about 1000 nm, about 400 nm to about 1000 nm, about 500 nm to about 1000 nm, about 600 nm to about 1000 nm, about 700 nm to about 1000 nm, about 800 nm to about 1000 nm, about 900 nm to about 1000 nm, about 1 nm to about 900 nm, about 1 nm to about 800 nm, about 1 nm to about 700 nm, about 1 nm to about 600 nm, about 1 nm to about 500 nm, about 1 nm to about 400 nm, about 1 nm to about 300 nm, about 1 nm to about 200 nm, about 1 nm to about 100 nm, about 1 nm to about 10 nm and about 10 nm to about 300 nm. The pores may be substantially uniformly distributed in and along the length of the fiber.

Depending on the size of the biological material to be encapsulated, the average pore size of the pores may be adjusted accordingly. The pore size should not be too small to prevent the movement of the contaminants in and out of the fiber. However, the pore size should not be too large such that the encapsulated biological material can escape easily from the fiber. The biological material may be a particle having a particle size in the nano-sized

range or in the micron-sized range. Hence, the biological material particle may have a diameter or equivalent diameter in the range of about 5 nm to about 10,000 nm or about 20 nm to about 5,000 nm. The pore size of the fiber
5 may be adjusted by adjusting the amount of humidity of the surrounding air during the electrospinning process, the amount, molecular weight or size of the pore-forming material added to the electrospinning mixture, the molecular weight of the polymer making up the fiber. The
10 pore size of the fibers may also be altered by varying the conditions of the electrospinning process such as voltage, tip to collector distance, flow rate, temperature etc.

The porous fiber may be an electrospun fiber. The diameter of the fiber may be in the range of about 10nm to
15 about 1000 nm, forming a nanofiber, or the diameter of the fiber may be in the range of about 1000 nm to 10 μ m, forming a microfiber.

The porous fiber may have a core-shell structure or may have multiple cores and shells made from various
20 materials. The pores may be present in the core(s) and/or shell(s). The diameter of the multiple core(s) may be in the range of about 1 nm to about 9000 nm or about 10 nm to about 5000 nm. The thickness of the shell wall may be in the range of about 1 nm to about 5000 nm. The biological
25 material(s) may be disposed in the core(s) or may be disposed in the shell(s). Where two or more biological materials are encapsulated, each core and shell structure may have a different biological material therein. Advantageously, this may allow the co-existence of two
30 biological materials that cannot be placed together or that need to be separated from each other. For example, a bacteria and an enzyme that perform different functions but have an antagonistic effect on each other when placed together can be separated using the core-shell

configuration. Further, in bioremediation, a number of bacteria species can be used. Usually, there are antagonistic bacteria that perform a function and other bacteria that prevent them from performing their functions but which also carry out a useful function. Hence, by separating these bacteria using the core-shell configuration, this can ensure that these bacteria are separated by a physical barrier and are not impeded from carrying out their individual functions. The shell(s) may act as a support for the core(s). The shell(s) may include nutrients for the biological material(s). The shell(s) may also act as a physical barrier to shelter the biological material present in the core(s) from attack by harmful predatory bacteria and higher organisms.

The biological material may be selected from the group consisting of bacteria, viruses, algae, fungi, enzymes, cells, proteins and nucleic acids.

When the porous fiber is used in bioremediation, the biological material may be a bacteria, enzyme, yeast, algae or a fungus. The bacteria, enzyme, yeast, algae or fungus may be chosen for their bioremediation properties in which they may be able to degrade contaminants that are present in the environment. These bacteria, enzyme, yeast, algae or fungus may be used in water, air and/or soil treatments for the biodegradation and removal of waste organic materials and other contaminants. For example, the bacteria, enzyme or fungus may be used during oil spills where the bacteria, enzyme or fungus is able to act on the oil and degrades it such that the oil contaminant no longer exerts a harmful effect on the environment. In another example, the bacteria or fungus may be able to degrade and reduce the level of heavy metals, chlorinated compounds, nitrates or other contaminating organic compounds in the environment. Yeast

such as *Yarrowia lipolytica*, may be used to degrade palm oil mill effluent and other hydrocarbons such as alkanes, fatty acids, fats and oils. Algae may be used to remove heavy metals and other ionic compounds from water such as nitrogen and phosphorus containing compounds.

The biological material may also be used in industries such as fermentation, catalysis of chemical reactions (in which enzymes are the common biological materials to use here), food processing (in which enzymes are the common biological materials to use here), paper industries, biofuel industries, medical industries (such as the use of encapsulated viruses as bacteriophages in phage therapy).

The bacteria may include, but is not limited to, *Bacillus Licheniformis*, *Bacillus Subtilis*, *Bacillus Amyloliquirefaciens*, *Bacillus Polymyxa*, *Bacillus lentus*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus sphaericus*, *Bacillus licheniformis*, *Acinetobacter haemolyticus*, *Acinetobacter baumannii*, *Brevibacillus brevis*, *Listeria seeligeri*, *Alcaligenes faecalis* type II, *Escherichia Hermanii*, *Bacillus Cereus*, *Bacillus Thuringiensis*, *Bacillus Meg'afarium*, *Corynebacterium*, *Brevibacterium Job 5*, *Alcaligenes entrophus*, *Pseudomonas Aeruginosa*, *Pseudomonas Statzeri*, *Pseudomonas Fluorescent*, *Pseudomonas Oleovorans*, *Pseudomonas putida*, *Pseudomonas desmolyticum*, *Pseudomonas methanica*, *Micrococcus paraffinae*, *Acetobacter peroxydans*, *Mycobacterium smegmatis*, *Mycobacterium thodoichrous*, *Achromobacter agile*, *Achromobacter centropunctatum*, *Arthrobacter*, *Bacillus hexacarbovorum*, *Nocardia opacus*, *Nocardia corrallina*, *Actinomyces oligocarboophilus*, *Desulfovibrio desulfuricans*, *Alcaligenes eutrophus*, *Nitromonas sp.* and *Rhodopseudomonas palustris*. The

concentration of the bacteria is dependent on the amount of contaminant to be degraded.

The algae may be both freshwater and marine algal species from the major classes of cyanobacteria, rhodophyta, chlorophyta, dinophyta, chrysophyta, prymnesiophyta, bacillariophyta, xanthophyta, eustigmatophyta, raphidophyta, phaeophyta. Specific examples include *Chlorella sp*, *Scendesmus sp*, *Chlorococcum sp*, *Ulothrix sp*, *Pseudanabaena sp* or *Haematococcus sp*.

Exemplary enzymes that are used in bioremediation include, but are not limited to, mono-oxygenases, dioxygenases, reductases, dehalogenases, cytochrome P450 monooxygenases, enzymes involved in lignin metabolism e.g. laccases, lignin-peroxidases, manganese-peroxidases. Bacterial phosphotriesterases. Other examples could also be molecularly engineered enzymes that are being produced.

The fungus may include, but is not limited to, *Candida lipolytica*, *Candida tropicalis*, *Candida utilis*, *Candida parasilosis*, *Candida guilliermondii*, *Candida rugosa*, *Trichosporan cutaneum*, *Aureobasidium pullulans*, *Pichia spartinae*, *Pichia saitoi*, *Torulopsis magnoliac*, *Torulopsis gropengiesseri*, *Trichoderma harzianum*, *Aspergillus versicolor*, *Penicillium sp* and *Graphium ulni*. The yeast may be *Yarrowia lipolytica*. The concentration of the fungus or yeast is dependent on the amount of contaminant to be degraded.

The contaminant may be an organic compound such as aliphatic hydrocarbons (for example, straight or branched chain hydrocarbons having a chain length ranging from about C₅-C₃₆ or ring structures of saturated C₅-C₃₆ hydrocarbons) and aromatic hydrocarbons (for example, at least one unsaturated ring structure ranging from C₉-C₂₂ hydrocarbons). Examples of such contaminants include, but are not limited to, dioxins, tars, creosote, crude oil,

refined oil, fuel oils (for example, Nos. 2, 4, and 6 fuel oils), diesel oils, gasoline, hydraulic oils, kerosene, chrysene, cresol, cyclohexanone, ethylbenzene, butylbenzene, ethyl acetate, fluorine, isoprenoids, methyl
5 ethylacetate, 2-butanone, methyl pentanone, methyl propylacetate, butylacetate, petroleum oils and greases, phenanthrene, trimethylbenzene, phenol, benzene, toluene, ethylbenzene, xylene, Stoddard solvents, mineral spirits, naphthalene, anthracene, acenaphthene, acenaphthylene,
10 benzo(a)anthracene, benzo(a)pyrene, benzo(b) fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, pyrene, terpene-based compounds, phthalates such as bis(2)ethylhexylphthalate and/or dioctylphthalate, and/or phenolic compounds.

15 The contaminant may be a halogenated aliphatic or aromatic compound such as a chlorinated aliphatic or chlorinated aromatic compound. Specific chlorinated hydrocarbon contaminants include, but are not limited to, polychlorinated biphenyls, aldrin, trichloroethylene,
20 tetrachloroethylene, 1,2-dichloroethane, carbon tetrachloride, chlorobenzene, chlorotoluenes, dichlorobenzene, dichloroethanes, dichloroethylene, dichlorotoluene, tetrachloroethane, trichloroethane, pentachlorophenol and vinyl chloride, etc.

25 The contaminant may be a nitrogen containing compound such as, but not limited to, an organic nitrogen containing compound and an inorganic containing compound. The organic nitrogen containing compound may be ammonia, ammonium or other materials that contribute to the Total
30 Kjeldahl Nitrogen in waters. The inorganic containing compound may be Group Ia and Group IIa metal nitrates or nitrites. The nitrate compound may be lithium nitrate, sodium nitrate, potassium nitrate, magnesium nitrate, calcium nitrate or ammonium nitrate. These nitrogen

containing contaminants may be removed by microalgae, nitrification and denitrification bacteria encapsulated within the fibers.

The contaminant may be phosphorous containing
5 compound, but not limited to, orthophosphates, polyphosphates, and organically bound phosphates.

The contaminant may be a metal such as, but not limited to, a transition metal, a rare earth metal, a metallic element from Group IIIa, IVa, Va and VIa of the
10 Periodic Table of Elements. The metal may be, but is not limited to, cadmium, zinc, cobalt, copper, lead, mercury, thallium, chromium and manganese in the form of salts, either in a soluble or non-soluble state.

During bioremediation, the nanofibers or microfibers
15 may be collected on a membrane support to form a membrane. The membrane support may be a polyester nonwoven support. The nanofibers or microfibers may be collected for a sufficient period of time until a desired membrane thickness is obtained. The thickness of the membrane may
20 be the same as the diameter of the nanofiber or the microfiber or may be up to until about 300 microns. If a thicker membrane is desired, individual layers can be stacked together. The membrane can be prepared as individual layers or stacked nanofiber or microfiber
25 layers on a support or may be stacked between two substrate materials. The substrate material may be any polymeric or inorganic material as is known to a person skilled in the art and may comprise openings for introducing a fluid to the membrane layer. The membrane
30 may be incorporated into modules such as flat sheet, tubular, spiral wound, or hollow fibers as required. The membrane may also be placed in a membrane housing to form a bioreactor or contactor. Hence, the bioreactor may comprise a membrane module in which the membrane module

comprises a plurality of porous fibers, each porous fiber encapsulating a biological material therein. The membrane contactor may comprise a membrane module whereby the membrane module comprises a plurality of porous fibers, 5 each porous fiber encapsulating a biological material therein.

When used as membrane contactors or reactors, the material to be acted upon or degraded by the biological material may be a chemical compound. This chemical 10 compound may be an inorganic compound or an organic compound such as organic waste, enzymes (that are used for pharmaceutical production, protein and DNA purification), food product, starch, glucose, etc.

Since the bioreactor already contains a certain 15 concentration of encapsulated biological material such as bacteria, yeast, algae or fungi, the bioreactor avoids the disadvantage of long start-up time, which can range from a few days to months, associated with a conventional bioreactor in which the bacteria, yeast, algae and/or 20 fungi needs time to grow until a certain concentration is reached for contaminant removal. Accordingly, the disclosed bioreactor can be used directly on a contaminated site in which the encapsulated bacteria, yeast, algae and/or fungi can act quickly on the 25 contaminants.

Further, due to the pores present in the nanofiber or microfiber, the pores offer a greater contact area between the encapsulated bacteria, yeast, algae and/or fungi with the contaminants. Hence, membrane fouling problems 30 associated with the build-up of contaminants on the surface of a membrane in a conventional bioreactor is minimised in the disclosed bioreactor because the encapsulated bacteria, yeast, algae and/or fungi can act directly on the contaminants. As the contaminants are

degraded and hence removed, the source of the membrane fouling is thus removed, leading to a decrease in the membrane fouling problem. Another instance whereby the membrane fouling problem in membrane bioreactors is reduced considerably is by the encapsulation of materials and compounds in the membrane that are of anti-quorum sensing nature, which may be from natural products or synthetically produced. These will prevent the attachment of biofilms on the membrane surface, which is a serious problem in membrane applications worldwide. The anti-quorum sensing materials may include, but is not limited to, enzymes from bacteria, furanone, halogenated furanone, red alga *Delisea pulchra*, southern Florida seaweeds, terrestrial plants, acylase, compounds containing lactone and other poly-hydroxylated rings.

When the porous nanofiber or microfiber is used as a biosensor, the biological material may be an enzyme, a cell, a protein or nucleic acid. When the biosensor is to be used to detect pathogenic organism in food stocks and supply chain, the biosensor can be prepared from porous fibers with dye encapsulating liposomes tagged with antibodies that are specific for the bacteria placed in the core of the fibers. These fibers can be formed into fiber mats or membranes. The mats or membranes can be in strip-form or swab-form. In another embodiment, when the biosensor is to be used to detect glucose and heavy metals, the biological material encapsulated in the fiber may be glucose oxidase. In yet a further embodiment, the biosensor may be comprised of a *LuxAB*-based material in which the *Lux* gene is a gene that is responsible for the light emitting reaction and which has been isolated from bacteria. This biosensor can then be used for, but not limited to, testing of antibiotic effectiveness, antimicrobial agents, bacterial biofilms, bacterial

biomass, bacterial stress response, bacterial transport mechanisms, bioremediation process monitoring, cell viable counts, circadian rhythms, DNA damaging agents, environmental contaminants, foodborne pathogens, gene
5 expression/regulation, growth phase regulation, immunoassays, industrial waste runoff, metabolic regulation, mutagenicity tests, plant pathogens, toxicity assays, viral infection, xenobiotic detection. In yet a further embodiment, the biosensor may be comprised of a
10 luxCDABE-based material that is used to detect 2,3 Dichlorophenol, 2,4,6 Trichlorophenol, 2,4-D, 3-Xylene, 4-Chlorobenzoate, 4-Nitrophenol, Aflatoxin B1, Alginate production, Ammonia, BTEX (benzene, toluene, ethylbenzene, xylene), cadmium, chlorodibromomethane, chloroform,
15 chromate, cobalt, copper, hydrogen peroxide, iron, isopropyl benzene, lead, mercury, N-acyl homoserine, naphthalene, nickel, nitrate, organic peroxides, PCBs, p-chlorobenzoic acid, p-cymene, pentachlorophenol, phenol, salicylate tetracycline, trichloroethylene,
20 trinitrotoluene, zinc, ultrasound, ultraviolet light, heat and gamma radiation.

The biological material may be a virus. The virus may include, but is not limited to, T7, T4 bacterial viruses, Herpes simplex, Cytomegalovirus, Papilloma virus,
25 Adenovirus, Burkitt lymphoma virus, Arbovirus, Arenavirus, Epstein-Barr virus, Varicella virus, Coronavirus, Cocksackievirus, Eboli virus, Enterovirus, Hepatitis virus, Influenza virus, Marburg virus, Measles virus, Mumps virus, Polio virus, Rhinovirus, Rubella virus, Smallpox
30 virus, Rabies virus, or Rotavirus. The nanofiber or microfiber encapsulating a virus may be used for phage therapy (where the virus is a bacteriophage) or as a gene delivery vector.

In one embodiment, the biological material is selected from the group consisting of bacteria, viruses, , algae, cells and yeast.

In another embodiment, the biological material is
5 selected from the group consisting of enzymes, proteins and nucleic acids.

In one embodiment, the biological material may be immobilized on a solid substrate. This is particularly advantageous when the biological material comprises
10 enzymes, proteins and nucleic acids or other small biomolecules. Accordingly, in one embodiment, when the biological material comprises enzymes, proteins and nucleic acids or other small biomolecules the biomaterial may be immobilized on a solid substrate. In one
15 embodiment, the solid substrate may be selected from the group consisting of gold nanoparticles, microparticles and the like. In one embodiment, the biomaterial is covalently bonded to the solid substrate via reactive groups, for example, -NH₂, -COOH, -SH, -CHO, -OH, peptide,
20 carbamate linkages and the like which can covalently bind to enzymes, proteins and nucleic acids and the like.

In addition to the use of membrane bioreactor or membrane contactor in bioremediation, the membrane bioreactor or membrane contactor can also be used in the
25 production of foodstuffs and pharmaceuticals. For example, emulsion enzyme membrane reactors may be used for the production of significantly high enantiomeric excess of the (S)-naproxen acid (anti-inflammatory drugs) from racemic mixtures of (R,S)-naproxen methyl ester in
30 emulsion membrane reactors where currently lipase is entrapped by physical methods in polymeric membranes [Li, N., Giorno, L., and Drioli, E. (2003), Effect of immobilization site and membrane materials on multiphasic enantioselective enzyme

Another use of the fiber relates to the incorporation of quorum-quenching or quorum sensing compounds in the porous fibers which are then formed as a membrane. This results in the prevention or quick biofilm attachment on the membrane surface, respectively, depending on the process required. In some water and wastewater treatment processes, biofilm is required on the surface to carry out degradation of the waste, for example, in biological activated carbon or sand filtration. Here, membranes can be used as the surface on which biological materials grow and cling to and the water is passed through it where the contaminants are removed. In membrane processes where water is pushed through the membrane, the attachment of biofilm on the membrane surface is considered detrimental as it blocks the pores and forms a cake layer. The cake layer prevents water from flowing through the membrane, resulting in low flux (for example, in applications such as reverse osmosis processes, microfiltration or ultrafiltration). Here, the quorum quenching biological materials can be encapsulated to prevent the membrane from getting fouled with biofilm.

The porous fiber may be an electrospun fiber. The electrospinning method will be discussed in detail further below.

The fiber may be formed from an electrospinnable polymer selected from the group consisting of a polyamide, a polyimide, a polycarbamide, a polyolefin, a polyurethane, a polyester, a polycarbonate, a polyaniline, a polysulfone, a polyacrylonitrile, a polycarbonate, a polyanhydride, a polyorthoester, a poly(acrylonitrile), a polybenzimidazole, a poly(siloxane), a polysilicone, a polycaprolactone, a polyhydroxyalkanoate, cellulose, copolymers, terpolymers and blends thereof.

The polymer may be selected from the group consisting of poly(vinylidene fluoride), poly(vinylidene fluoride-co-hexafluoropropylene), polyvinyl pyrrolidone, poly(N-vinyl pyrrolidone), polymethyl methacrylate, polyacrylic acid, 5 polyvinyl acetate, polyacrylamide, polyethylene, cellulose acetate, cellulose acetate butylate, polyvinyl pyrrolidone-vinyl acetate, poly(2-hydroxy ethyl methacrylate), polyethyleneimide, polyethersulfone, polystyrene, nylon, nylon12, nylon-6, nylon-6,6, nylon-10 4,6, aramid, polydimethylsiloxane, polyvinylchloride, poly(L-lactic acid acid-caprolactone), poly-L-lactic acid, polyvinylalcohol, polyethyleneimine, polyethylene oxide, poly(ethylene terephthalate), poly(p-phenylene terephthalamide), polytrimethylene terephthalate, 15 poly(hydroxyl butyrate), polyester urethane, polyether urethane, poly(propyleneoxide), poly(ethylene-co-vinyl acetate), poly(ethylene glycol), poly(methacrylic acid), polyglycolide, poly(lactide-co-glycolide), polyanhydride, polyvinyl carbazole, polyvinyl phenol, polystyrene, 20 polyhydroxyacids, polysulfones, polytetrafluoroethylene, polyacrylonitrile, polystyrene, copolymers, terpolymers and blends thereof.

In one embodiment, the fiber may be formed from chitosan, collagen or gelatin.

25 In an embodiment where the porous fiber has a core-shell structure, the above polymer may be independently selected as the material for the core and/or shell of the porous fiber. The same polymer may be used for both the core and the shell structures. In another embodiment, 30 different polymers may be used for the core(s) and shell(s) structures.

In one embodiment the polymer has a tensile strength selected from the group consisting of at least 10 MPa, at least 15 MPa, at least 20 MPa, at least 25 MPa, at

least 30 MPa, at least 45 MPa, at least 50 MPa and at least 60 MPa. In one embodiment the polymer has a tensile strength in the range selected from the group consisting of from about 10-60MPa, about 15-60MPa, about 20-60MPa, about 25-60MPa, about 30-60MPa and about 35-60MPa, about 40-60MPa, about 45-60MPa, about 50-60MPa and about 55-60MPa. In one embodiment, the polymer has a tensile strength of about 52MPa.

In one embodiment the polymer has a tensile modulus selected from the group consisting of at least 400 MPa, at least 450MPa, at least 500MPa, at least 550 MPa, at least 600 MPa, at least 650 MPa, at least 700 MPa, at least 750 MPa, at least 800 MPa, at least 850 MPa, at least 900 MPa, at least 950 MPa, at least 1000 MPa, at least 1100 MPa, at least 1200 MPa, at least 1300 MPa, at least 1400 MPa, at least 1500 MPa, at least 1600 MPa, at least 1700 MPa. In one embodiment the polymer has a tensile modulus in the range selected from the group consisting of about 1000-1800MPa, about 1100-1800MPa, about 1200-1800MPa, about 1300-1800MPa, about 1400-1800MPa, about 1500-1800MPa, about 1600-1800MPa and about 1700-1800MPa. In one embodiment, the polymer has a tensile modulus of from about 1723MPa. In one embodiment, the polymer is poly(vinylidene fluoride) (PVDF). In one embodiment, the polymer is a non-biodegradable polymer.

The use of PVDF is advantageous as this polymer is mechanically strong and as it is non-biodegradable it is ideal candidate for applications such as waste water treatment. In comparison, for example, fibers made of the polymer polycaprolactone (PCL), which is a biodegradable polymer, are significantly weaker than fibers made from PVDF. Additionally, biodegradable polymers, such as PCL, are susceptible to degradation by the action of aerobic and anaerobic microorganisms that are widely distributed

in various ecosystems (Tokiwa, 2009). As such, biodegradable polymers can be easily degraded by lipases and esterases and thus are not suitable for use with microorganisms such as bacteria or viruses.

5 The method of forming the porous fiber having a core-shell configuration, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material may comprise the steps of: providing a core solution of the biological material; providing a mixture
10 comprising a fiber material and a pore-forming material as the shell solution, wherein the fiber material and the pore forming material are miscible with each other; forming a core-shell fiber from said core solution and shell solution, said fiber encapsulating and retaining
15 said biological material in the core; and removing said pore-forming material from said formed core-shell fiber to create pores therein.

 The fiber may be formed using an electrospinning step. Electrospinning is a known method in the art and
20 typically involves an electric charge to pull a liquid jet of a viscous solution from the tip of a spinneret. The electrospinning technique may employ a core-shell or coaxial electrospinning technique, or an equivalent method known in the art, in which two solutions, one of which
25 forms the inner core and the other forms the outer shell. A spinneret is used to contain the core or shell solution. The spinneret can be further modified to contain several cores or several shells of varying materials and properties.

30 During electrospinning, an electric field is applied to the droplet by connecting a first electrode to the tip of the spinneret which is made from a conducting material. A counter-electrode is placed at a selected distance from the tip of the spinneret and a high voltage current of

from 1 to 30 kV is applied. The electrode may be formed from any suitable material, such as copper or any other conducting material. The distance between the tip of the spinneret and the counter-electrode, known as the tip to collector distance, is kept between 5 and 30 cm. The spinneret may be stationary or moveable in all directions as required for the deposition of the fibers.

When the electrostatic field applied is greater than the surface tension of the viscous solution, a nanofiber jet is emitted from the tip of the spinneret. Electrostatic forces associated with mutual Coulombic interactions at different sections of the jet make it unstable when subjected to bending perturbations. The bending instability rearranges the jet into a sequence of connected loops, which becomes unstable and forms secondary and tertiary loops, leading to the stretching of the fibers to form a smaller fiber.

A collector is placed under the tip of the spinneret. The collector may comprise, for example, a rotating collector drum or a moveable plate. The collector drum or plate may be formed of any suitable material, such as aluminium, zinc, or any other conducting material. As mentioned above, a membrane support material may be used as the collector.

The core solution may be a mixture comprising a fiber material, a pore-forming material and a biological material. The core solution may be introduced into the core chamber of the spinneret. The shell solution may comprise the same fiber material as the core or may comprise a different fiber material compared to that in the core. The shell solution may also comprise an inorganic material. The shell solution may comprise a pore-forming material. Hence, the pore-forming material may be present in one of the core solution and shell

solution or be in both the core and shell solutions. The fiber material in the core and/or shell may be an electrospinnable polymer as mentioned above.

5 The biological material may be suspended in an aqueous or non-aqueous buffer such as a phosphate buffer solution.

The core solution may further comprise an osmolarity regulating agent selected from the group consisting of glycerol; glycol; polyethylene glycol; sugar such as
10 sucrose, glucose, fructose, lactose, etc; sugar-alcohol such as mannitol, inositol, xylitol, and adonitol; amino acids such as glycine and arginine; biological polymeric molecules and proteins such as albumin; as well as Ficoll®.

15 The core solution may further comprise additives to increase or maintain the viability of the biological materials. The additive may be a nutrient selected from one or more of the following carbohydrates (such as glucose, fructose, maltose, sucrose, and starch); other
20 carbon sources (such as mannitol, sorbitol and glycerol); nitrogen sources (such as urea, ammonium salts, amino acids or crude proteins, yeast extract, peptone, casein hydrolysates and rice bran extracts); and inorganic compounds (such as magnesium sulfate, sodium phosphate,
25 potassium phosphate, sodium chloride, calcium chloride and ammonium nitrate). The core solution may further comprise additives to increase the activity of the biological material such as vitamins or nutrients.

The shell solution may comprise an electrospinnable
30 polymer as mentioned above. The shell solution may further comprise a ceramic precursor. The shell solution may comprise the osmolarity regulating agents and/or additives as mentioned above. The shell solution may include inorganic material such as metallic or metallic

oxide nano-materials that increase the strength of the fibers or increase the likelihood of interaction between the contaminants in the water, air or solid waste to the fibers so that they can be treated or interact better with the entrapped biological materials.

The electrospinnable polymer may be dissolved in a suitable solvent for the polymer. The solvent for the shell solution is selected to have low solubility with the solvent used in the core solution. The solvent in the core solution may be water, ethanol, or other solvents that are somewhat incompatible with the solvents used in the shell. The core solvent should be carefully chosen because if it is not compatible with the polymer in the shell solution, clogging of the needle during electrospinning may occur, which require cleaning of the needle tip by wiping it with a cloth or string. It is to be appreciated that a person skilled in the art would know the type of solvent that is suitable for the core and shell solution. A suitable solvent for the shell solution may include, but is not limited to, hexane, cyclohexane, dimethylformamide, acetone, acetonitrile, hexafluoroisopropanol, dimethylacetamide, formic acid, ethanol, methanol, toluene, m-Cresol and trifluoroethanol. The solvent may be a blend or a combination of solvents.

In one embodiment, the boiling point of the solvent may be in the range of about 30°C to about 200 °C. In another embodiment, the pore-forming material may be a low boiling point solvent. The boiling point of the solvent may be in the range of about 30°C to about 80 °C. The low boiling point solvent may include, but is not limited to, chloroform, acetone, ethyl ether, benzene, cyclohexane, toluene, dimethylformamide, tetrahydrofuran, hexafluoro-2-propanol, trifluoroethanol and other suitable solvents that have a boiling point that falls within the above

range and can be electrospun. Due to its low boiling point, the solvent evaporates from the formed fiber, resulting in pores being formed in the fiber. If the biological material used is one that can withstand a high temperature, it may be possible to apply heat to accelerate the evaporation of the low boiling point solvent from the fiber and to control the pore structure. Another method to form pores in the fiber is to soak the fibers after preparation into a non-solvent of the shell polymer, for example, water which has a higher density than that of the solvent used in the shell. This will cause phase separation and the formation of pores on the shell of the fiber. A further method to remove the low boiling point solvent is to increase the relative humidity of the surrounding air. For example, when the low boiling point solvent used is one of toluene, hexafluoro-2-propanol, trifluoroethanol or dimethylformamide (that has a high vapour pressure) that is electrospun with hydrophobic polymers such as polystyrene, polyvinyl chloride or poly(methyl methacrylate) in the shell, pores are formed when the relative humidity of the surrounding air is higher than 30%.

The pore-forming material may be a polymer that is soluble in a solvent, for example an aqueous medium. After the fiber is formed, the fiber may be placed in a solvent such as water to allow the polymer to dissolve. As the polymer dissolves, pores are formed in the core-shell fiber. The polymer may include, but is not limited to polyacrylamide, polyvinyl alcohol, polyacrylic acid, poly(ethylene oxide), methyl cellulose, hydroxyethyl cellulose, carboxymethyl cellulose, poly(allyl) amine, poly(diallyl dimethyl ammonium chloride), poly(diallyl methyl amine hydrochloride), polymethacrylic acid, sodium polyacrylate, polyvinylbenzyl trimethylammonium chloride,

poly(sodium-2-sulfoethylacrylate), polyvinylbenzyl sodium sulfonate, poly(sodium styrene sulfonate), polystyrene sulfonate, poly(dimethylaminoethyl methacrylate), poly[(methacrylamido)propyltrimethylammonium chloride],
5 polyethylene glycol and poly(acrylonitrile).

The pore-forming material may include an inorganic, water soluble material such as water soluble salts, sugars, nanoparticles, nanomaterials and crystals which can be incorporated in the shell matrix and removed by
10 extended soaking in water. The water soluble salt may be a water soluble metallic salt such as Iron Salts, Lithium Salts, Calcium Salts, Sodium Salts, Magnesium Salts, Crystals, nanoparticles or microparticles of these materials, to generate larger pores.

15 The pore-forming material may be a sacrificial template which can be organic or inorganic in nature. The sacrificial template is added together with the biological material, electrospun and subjected to an additional step to remove the template in a non-surfactant template
20 technology. For example, urea molecules may be used as a non-surfactant template or pore-forming agent. The urea may be removed by soaking the fiber in water or methanol. Similarly, glucose, sucrose or other larger molecular weight water soluble materials may be used.

25 In another embodiment, the pore-forming agent may be a blend of non water soluble polymers with the water soluble polymers as mentioned above.

The pore-forming material may be a mixture of the low boiling point solvent, the soluble polymer, the inorganic
30 material and sacrificial template.

The pore size of the pores may be adjusted by controlling the concentration, molecular weight or size of the pore-forming material, the humidity of the electrospinning chamber, the flow rate of the electrospun

solutions and the diameter of the formed fiber. The concentration may affect the porosity of the fibers as the higher the concentration, the greater the number of pores and/or the bigger the pore size. The size of the pore-forming material in the form of a water soluble material and the amount removed will affect the pore size. The bigger the size and amount removed would lead to bigger pores formed. If a low boiling point solvent is used as the pore-forming material, humidity of the electrospinning chamber is a factor that affects the formation of the pores. For example, if the humidity is below 20%, pores are generally not formed. Conversely, the higher the humidity, the bigger the pore sizes. In addition, another factor that can affect the pore size when a low boiling point solvent is used as the pore-forming material is the diameter of the fiber. The fiber diameter should be larger than about 500 nm for pore formation. At smaller diameters, not many pores are visible on the fiber. Generally, the bigger the diameter of the fiber, the more pores are formed and the bigger the pores are. Therefore, factors that can affect the diameter of the fibers are injection rate of the core and shell solutions, the applied voltage, the tip to collector distance and the polymer concentrations.

Brief Description Of Drawings

The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig. 1 is a schematic diagram of the equipment used during electrospinning.

Fig. 2 is a graph showing the percentage conversion of glucose by the yeast encapsulated membrane after soaking over a number of days.

Fig. 3 is a high resolution microscope image at 100 X magnification of a porous fiber encapsulating an algae *Chlorella Sp.*

Fig. 4 is a SEM image at 13260 X magnification showing a porous fiber made from Polyimide

Fig. 5 is a SEM image of thin PCL nanofibers of thickness less than 200nm at (a) low and (b) high magnification under SEM.

Fig. 6 is a high magnification SEM image of PCL nanofiber using 23% PCL nanofibers showing thick nanofibers.

Fig. 7 is a SEM image of the pores produced by using different concentrations of Porogen (a) 20mg/ml and (b) 30mg/ml.

Fig. 8 shows the high magnification SEM images of PCL nanofibers without (a) and with (b) Porogen.

Fig. 9 shows the confocal images of core-shell nanofibers of PCL with Porogen (a) overlay image (b) X Y section image.

Fig. 10 shows the SEM high magnification images of nanofibers electrospun using 15% PVDF solution in Dimethyl Acetamide and Acetone.

Fig. 11 shows the SEM high magnification images of rough nanofibers electrospun using 20% PVDF solution in Dimethyl formamide and Water (30:1)

Fig. 12 shows the SEM high magnification images of nanofibers electrospun using PVDF+ PEO (45: 8): DMF+ Water (30: 1) 20% ratios, showing pores on thick fibers and beads only.

Fig. 13 shows the SEM pictures of nanofibers prepared by using A) Higher concentration (25%) of PVDF: PEO (45:8) in DMF and Water B) High polymer: Porogen ratio (40:15)

Fig. 14 shows SEM pictures of PVDF nanofibers with various thickness using different percentages of polymer for spinning.

Fig. 15 shows the Optical (A) and fluorescent (B) images of nanofibers PVDF: PEO as shell and Glycerol: Concavalin A- Alexa Fluor as core.

Fig. 16 shows the confocal images of porous core-shell nanofibers prepared by using PVDF and Porogen.

Fig. 17 shows the SEM images showing thick highly porous nanofibers of electro-spun Poly(caprolactone) with THF and DMSO.

Fig. 18 is schematic illustration of the bioconjugation of nanoparticles with different ligands.

Fig. 19 is a schematic to show the immobilization of biomolecules on the nano/micro particles and beads using covalent functionalization.

Detailed Description of Drawings

Referring to Fig. 1, there is provided a schematic diagram of the equipment used during electrospinning. The coaxial or core-shell spinneret 2 consists of an internal chamber 4 and an external chamber 6. The core solution denoted by the dotted line 10 is pumped from a core solution holder 8 into internal chamber 4. The shell solution denoted by the dotted line 14 is pumped from a shell solution holder 12 into internal chamber 6. The core solution holder 8 and shell solution holder 12 may be a pipette or a syringe. If syringes are used, the core and shell solution are introduced into the respective chambers in the spinneret 2 via a syringe pump 16.

For the spinneret 2, there are no requirements for the volume of the internal chamber 4 and external chamber 6. However, the internal chamber 4 and external chamber 6 should be fabricated such that there is no mixing of the core and shell solutions except at the tip 20 of the spinneret 2. The tip 20 of the spinneret 2 may be a pipette tip or a needle. The diameter near the tip 20 may have an inner diameter of about 0.05 to 3 mm. The speed by which the core solution and shell solution exit the spinneret 2 via the tip 20 is regulated by the speed of the syringe pump 16. The speed of the core solution and shell solution can be independently controlled so that the speed of the core and shell solution can be different from each other.

The core solution is a mixture of the electrospinnable polymer and the biological material. As mentioned above, the core solution may contain additives such as an osmolarity regulating agent or nutrients. As mentioned above, the shell solution may include an electrospinnable polymer, optionally mixed with one of an inorganic material and a pore-forming material.

An electrostatic field 18 is applied to the spinneret 2. The electrostatic field 18 can be generated between a first electrode (not shown) and a second counter-electrode (not shown). The first electrode is inserted in the spinneret 2 and the second electrode is positioned at a distance of about 5 to 30 cm from the first electrode. A high voltage of about 1 to 30 kV is applied between the first and second electrodes to generate the electrostatic field 18.

As the electrostatic field 18 is applied to the spinneret 2, when the electrostatic field 18 applied is greater than the surface tension of the solutions in the spinneret 2, a nanofiber jet 22 is emitted from the tip 20

of the spinneret 2. The nanofiber jet 22 is then propelled towards a collector 24 which can be a rotating disc. The second electrode can either be connected to the collector 24 or it can be the collector 24.

5

Examples

Non-limiting examples of the invention and a comparative example will be further described in greater detail by reference to specific Examples, which should not
10 be construed as in any way limiting the scope of the invention.

Example 1

Materials

15 Nylon 6/6, Polyvinyl Alcohol (MW 85-124kDa) and Polyethylene glycol (Mn 570-630) were purchased from Sigma Aldrich of St. Louis of Missouri of the United States of America. Formic acid (98-100 %) and D (+) Glucose were obtained from Merck. Bacto Peptone and Backers yeast
20 (*Saccharomyces cerevisiae*) were purchased from Becton Dickinson of Franklin Lakes of New Jersey of the United States of America and Gim Hin Lee Pte Ltd (Singapore) respectively.

25 Methods

a) Preparation of core-shell nanofiber

A 15 % (w/w) Nylon spinning solution was prepared by dissolving Nylon 6/6 in a mixture of formic acid and Polyethylene Glycol (14:3 w/w) and used as shell solution.
30 Baker's yeast pellets (*Saccharomyces cerevisiae*) was resuspended in deionized water and centrifuged at 7500 rpm. The residue was further resuspended in 3 ml water and used as the core solution. The shell solution was injected into the outer coaxial needle and the core solution was

delivered into the inner coaxial needle at a constant rate of 3 ml/hr and 1 ml/hr, respectively, by using a programmable syringe pump. A positive high-voltage supply of 23 kV was maintained between the spinneret and the
5 metallic grounding plate and fibers are collected up on the plate. The tip to collector distance was maintained at 100 mm. The nanofibrous mat obtained was then soaked in deionized water for 15 minutes with shaking and washed for three times with deionized water.

10 A control membrane of the same formulation above for the core (except the yeast particles) and shell solutions was also prepared under the same operating conditions. All prepared membranes had a thickness of approximately 40 μm and were cut into circular coupons of 50 mm diameter for
15 use in the assay of yeast activity. The weight of the membrane was approximately 1.3 g.

b) Assay of Yeast activity

The core-shell nanofibrous mat prepared was incubated
20 in an aqueous media containing 0.8 % (w/v) Glucose and 0.2 % (w/v) Peptone. A quantitative analysis of Yeast activity was done by measuring the glucose concentration spectrophotometrically at 575 nm by using DNS as the color indicator (1). A decrease in glucose concentration
25 directly indicates yeast activity since yeast can metabolize glucose into ethanol and carbon dioxide. The glucose concentrations in the control membrane and the yeast encapsulated membrane are shown in Table 1 below.

Membrane	Concentration of Glucose Remaining (mg/mL)			
	Day 1	Day 2	Day 5	Day 7
Control Membrane	0.7265	0.7265	0.7260	0.7269
Yeast Encapsulated Membrane	0.7265	0.5481	0.5180	0.1564
Percentage change (%)	0	24.6	28.7	78.5

Table 1: Concentration of glucose remaining in the various membranes

The percentage change in glucose concentration due to the yeast encapsulated membrane is shown in Fig. 2.

The yeast encapsulated membrane and control membrane were removed at the end of 7 days and washed in deionised water. The washed membranes were then placed into freshly prepared glucose solution having an initial concentration of 0.83 mg/mL. The glucose concentrations of the vials containing the membranes were then monitored. It was found that the yeast encapsulated membrane was able to convert 4% and 25.9% of glucose after Day 2 and Day 4, respectively, of this experiment, which corresponded to Day 9 and Day 11, respectively, of testing from the time the membranes were first produced. The results are encouraging for the extended use of the encapsulated membranes.

Example 2

Methods

a) Preparation of Core-shell nanofiber

A 15 % (w/w) Nylon spinning solution was prepared by dissolving Nylon 6/6 in a mixture of formic acid and Polyvinyl alcohol (PVA) (14:3 w/w) and used as shell solution. Baker's yeast pellets (*Saccharomyces cerevisiae*) were resuspended in deionized water and

centrifuged at 7500 rpm. The residue was further resuspended in 3 ml water and used as the core solution. The shell solution was injected into the outer coaxial needle and the core solution was delivered into the inner coaxial needle at a constant rate of 3 ml/hr and 1 ml/hr, respectively, by using a programmable syringe pump. A positive high-voltage supply of 23 kV was maintained between the spinneret and the metallic grounding plate and the fibers were collected on the plate to form a nanofibrous mat. The tip to collector distance was maintained at 100 mm. The nanofibrous mat obtained was then soaked in deionized water for 15 minutes with shaking and washed thereafter for three times with deionized water.

A control membrane of the same formulation above for the core and shell solutions but without the yeast particles was prepared under the same operating conditions.

A total of three yeast encapsulated membranes and three control membranes were cut into circular coupons. The mass of each pair of yeast encapsulated membrane and control membrane was 0.27, 0.35 and 0.53 g. The yeast encapsulated membranes and control membranes were used in the assay of yeast activity.

25

b) Assay of Yeast activity

Each of the core-shell nanofibrous mat prepared was incubated in an aqueous media containing glucose. A quantitative analysis of yeast activity was done by measuring the glucose concentration spectrophotometrically at 575 nm by using DNS as the color indicator (1). A decrease in glucose concentration directly indicates yeast activity since yeast can metabolize glucose into ethanol and carbon dioxide. The glucose concentrations in the

control membranes and the yeast encapsulated membranes after 4 days of soaking are shown in Table 2 below. The starting glucose concentration was 1.80 mg/mL.

Weight of Membranes (g)	0.27	0.35	0.53
Glucose concentration remaining in control membrane (mg/mL)	1.78	1.70	1.79
Glucose concentration remaining in yeast encapsulated membrane (mg/mL)	1.56	1.34	1.25
Percentage removal (%)	12.4	21.2	30.2

5 Table 2: Glucose concentration and percentage removal in the various membranes of different masses

Table 2 shows a positive relationship between the amount of glucose removed and the weight of the membrane.
 10 This is due to the greater amount of yeast particles present in the membranes of increasing weight.

Example 3

Algae *Chlorella sp* was encapsulated into a PVDF nanofiber. A 15 % (w/w) Poly vinylidene fluoride (PVDF) spinning solution was prepared by dissolving PVDF in a mixture of N,N Dimethyl acetamide and Acetone (2:3 w/w) and used as shell solution. The algae *Chlorella sp* suspended in deionized water was used as the core solution. The shell solution was injected into the outer coaxial needle and the core solution was delivered into the inner coaxial needle at a constant rate of 3 ml/hr and 1 ml/hr respectively by using a programmable syringe pump. A positive high-voltage supply of 16 kV was maintained
 25 between the spinneret and the metallic grounding plate and the encapsulated nanofibrous mat was collected on an aluminium plate. Due to the presence of acetone (a pore-forming material with low boiling point), as the mat was

collected on the aluminium plate, the acetone evaporated from the fiber to form pores on the fiber. Fig. 3 is a high resolution image at 100X magnification of the porous fiber encapsulating the *Chlorella Sp.*

5 The total diameter of the fiber was approximately 8000 nm. The *Chlorella sp* has a diameter of approximately 2500-3000 nm. Fig. 3 was obtained under a controlled flow rate to allow single algae cells to be deposited within the fibers. More cells can be deposited by adjusting the
10 flow rates, concentration of the core fluid and the electrospinning conditions.

Example 4

Fig. 4 is a SEM image of a polyimide fiber. This was made using the following process. A 25 % (w/w) Polyimide
15 spinning solution was prepared by dissolving Polyimide in a mixture of N,N Dimethyl formamide and Acetone (17:3 w/w). The spinning solution was injected through an 18 G needle at a constant rate of 2 ml/hr by using a programmable syringe pump. A positive high-voltage supply
20 of 20 kV was maintained between the spinneret and the metallic collector. The nanofibers were collected on to the surface of the metallic collector. Due to the presence of acetone (a pore-forming material with low boiling point), as the mat was collected on the aluminium plate,
25 the acetone evaporated from the fiber to form pores on the fiber. Fig. 4 shows the porous fiber that was collected.

Example 5

30 Materials

Polycaprolactone PCL (Mn45,000), poly(ethylene glycol) (PEG) (Mw 3000-37000) and fluorescein 5 (6) isothiocyanate (FITC) was purchased from Sigma Aldrich, Singapore. Ethanol (absolute) and glycerol was purchased

from Merck, Singapore and dichloromethane was obtained from J.T.Baker, United States of America.

Methods

5 Preparation of polymer solution

The shell solution was 23 % polycaprolactone (PCL) with 20 mg/ml or 30mg/ml of poly (ethylene glycol) (PEG) dissolved in a mixture of 60: 40 (v/v) dichloromethane: ethanol; the core solution was a small amount of
10 fluorescein isothiocyanate mixed with 20% glycerol.

Electrospinning Set Up

The spinning parameters were as follows: The flow rates of both core and shell solution were 1mL/h and 3
15 mL/h. The needle gauges used for dispensing core and shell solutions were 18G and 27 $\frac{1}{2}$ G. The shell solution was injected into an outer coaxial needle and the core solution was delivered into an inner coaxial needle. A positive high-voltage supply of 15 kV was maintained
20 between the spinneret and the metallic grounding plate and the fibers were collected on the plate to form a nanofibrous mat. The tip and collector distance was 100 mm. For confocal microscopy, fibers were collected directly onto a microscope slide. The nanofibrous mat
25 obtained was then soaked in deionized water for 24 hrs to dissolve the porogen PEG and dried overnight in desiccators prior to imaging under SEM. All the experiments were conducted at room temperature.

30 Results

The size and morphology of the PCL fibers was analyzed by scanning electron microscopy (SEM) and the core shell structure was confirmed by confocal microscopy. A concentration of 10% of PCL was used which produced

nanofibers having a thickness of less than 200nm (see Fig. 5a and b) which was not suitable for bacterial incorporation into the core. In order to increase the fiber size, another concentration of 23% was used which
5 produced thicker nanofibers in the range of 1 to 1.5 μm (see Fig. 6).

A porogen, PEG was used in different concentrations in combination with PCL to create the pores of different
10 sizes. It was found that when 20mg/ml porogen was used, the nanofibers showed pores of about 100nm size (see Fig. 7a). Whereas, when the porogen concentration was increased to 30 mg/ml, the pore size was increased up to 200nm (see Fig. 7b). Pore sizes were measured using high
15 magnification images obtained with SEM using Cell P Software.

No prominent changes either in the surface morphology or fiber diameter were observed in PCL fibers formed with
20 and without porogen. This is represented in Fig. 8 at different magnifications.

Fig. 9 shows the confocal image of the core shell fiber obtained. The solution mixed with FITC was used as a
25 core during spinning whereas only polymer solution was used as the shell solution without any fluorescence. Hence, the differential interference contrast microscopy (DIC) image is overlaid with a fluorescent image. The fluorescent material was excited with a laser and point by
30 point scanning was used to generate z stacking (layer-by-layer image).

However, the DIC image was obtained using the passage of light. As such, layer by layer scanning is not possible. Fig. 9 shows the confocal images obtained for

the fibrous mat collected after electrospinning. As the fibers are in different Z planes, fluorescence is not observed for all the fibers. The fibers which are at a particular plane of measurement will exhibit fluorescence.

5 The Z stacking is subjected to X and Y sectioning to prove the presence of fluorescent core inside the fiber. The presence of this feature proves that the fibers are capable of encapsulating biological material therein.

10 **Example 6**

Methods

a) Preparation of Electrospun PVDF Fibers

Porous poly(vinylidene fluoride) (PVDF) fibers were prepared by electrospinning from solutions in
15 dimethylformamide, poly(ethylene oxide) (PEO) and water.

Materials

Kynar poly(vinylidene fluoride) (PVDF) was provided by Arkema Inc., France. Poly(ethylene oxide) (PEO)
20 (average Mw 100,000), dimethylformamide (DMF), Dimethyl acetamide (DMAc), Dimethyl sulphoxide (DMSO) and Acetone were obtained from Sigma Aldrich, Inc. De-ionized water was used in the experiments for solution preparation. All materials were used without further purification.

25

Characterization of Porous Electrospun PVDF Fibers

For morphological characterization by scanning electron microscopy (SEM) (JEOL-JSM-5200, Japan), fiber samples were sputter-coated with a 10 nm layer of gold
30 using a magnetron sputtering auto fine platinum coater. SEM was used to observe the surface structure of fibers at 30 kV acceleration voltage and 10 mm working distance. Using the JEOL JSM-5200 SEM for image acquisition, pores smaller than 50 nm are easily overlooked due to the

limited resolution and quality of the SEM image. A "rough surface" is defined in this work as one having no obvious pores larger than 50 nm on the fiber surface.

5 Variations in PEO concentration, solvent nature and concentration were investigated as possible factors that may influence the development of pores on PVDF fibers electrospun with small amounts of PEO (additive) and water (non-solvent). All the experimental results are shown in
10 the following figures 10 to 17. The sample prepared using only PVDF in DMAC:Acetone was used as benchmark to demonstrate the influence of different factors on the final morphology of porous core-shell electrospun PVDF fibers.

15

Sample Preparation

PVDF and PEO were dissolved in mixtures of DMF and water, in the amounts shown in Table 3, under gentle stirring overnight at 70°C. The solutions were
20 subsequently cooled down to room temperature before Electrospinning. The flow rate, plate-to-plate distance, and voltage, respectively, were 2 mL/min, 15 cm, and 16 kV for the solutions. Nanofibers were collected on a grounded aluminum foil at room temperature. The nanofibrous mat
25 obtained was then soaked in deionized water for 24 hrs to dissolve the porogen PEG and dried overnight in desiccators prior to imaging under SEM. All the experiments are conducted at room temperature.

30 When 15% PVDF solution was prepared in Dimethyl Acetamide: Acetone in (1:4) ratio the nanofibers showed very smooth surface under high magnification SEM (Fig. 10).

Preparation of Porous Electrospun PVDF Fibers

A combination of Dimethyl formamide (DMF) and water were used as solvents for spinning the porous nanofibers. A porogen, poly (ethylene oxide) (PEO) was added to the polymer in order to produce pores on the nanofiber surface. A 20% PVDF and PEO (43:10) solution was prepared in DMF:Water (30:1). All other conditions were same as above for Electrospinning. The nanofibers showed a very rough surface under high magnification SEM but no pores were seen (see Fig. 11).

In order to get porous nanofibers, different ratios of PEO (Average Mw 100,000) and PVDF were tested as listed in Table 3 below using DMF and water. (Yang, 2011). PVDF and PEO were dissolved in DMF and water in amounts shown in Table 3 with continuous stirring at ~70C overnight. Solutions were allowed to cool down before electrospinning.

No	PVDF/PEO wt ratio	DMF/H ₂ O	Concentration n (PVDF+PEO) / (DMF+H ₂ O)	Result
1	50:3	30:1	5%	Can't spin
2	50:3	30:1	8%	Can't spin
3	50:3	30:1	12%	Can't Spin
4	50:3	30:1	15%	Can't spin, have beads
5	50:3	30:1	18%	Fibers have Beads
6	50:3	30:1	20%	Fibers have pores on beads only
7	50:5	30:1	20%	Can't spin
8	50:10	30:1	20%	Can't spin
9	43:10	30:1	20%	Fibers without pores, rough

				surface
10	48:5	30:1	20%	Fibers with pores on beads only
11	45:8	30:1	20%	Have pores on beads and thick fibers
12	45:8	30:1	25%	Thick fibers, pores on all fibers
13	43:10	30:1	25%	Thick fibers, pores on thick fibers
14	40:13	30:1	25%	Thick fibers, pores on thick fibers
15	40:15	30:1	25%	Thick fibers, bigger pores on thick fibers

Table 3: Different solution conditions for spinning to obtain porous nanofibers of PVDF.

5 Out of these different conditions listed in Table 3, most of the solutions could be electrospun. However, under some conditions pores were produced on beads only. When the total percentage (PVDF+PEO)/(DMF+H₂O) was increased from 20 to 25%, the thicker fibers were produced (~2μm) and showed pores on almost all spun nanofibers. In this case, no beads were observed. Attempts were also made to increase the pore size by increasing the PEO ratio and it was found from SEM images that the size of pores increased up to 200nm.

15

Fig. 12 shows nanofibers containing pores on only thick fibers and beads which have diameter of more than 2 μm. Thin fibers did not show any pores.

20

When the polymer concentration was increased to get thicker fibers in order to obtain pores on the majority of

spun fibers, it was found that using 25% PVDF and PEO in ratio of 45:8, thicker fibers up to 2 μ m could be obtained. These fibers showed well defined pores of the size around 100 nm (see Fig.13A) on the surface distributed uniformly everywhere. To further increase the size of nanofiber pores, the polymer:porogen ratio was further increased to 40:15 which obtained bigger pores of around 200nm (see Fig.13B).

Additionally, the nanofiber thickness could also be controlled by varying the polymer concentration used to spin the nanofibers. For example, a 15% solution obtained nanofibers have a thickness of less than 1 μ m where as this thickness was increased when polymer concentration was increased to 20% with a further increase with 25% solution. (Fig. 14)

Preparation of Core-Shell PVDF Electrospun Nanofibers

PVDF and PEO polymer (25%) solutions were used in DMF and water (30:1 ratio) to prepare the shell of the nanofibers and Glycerol was used as the core. These nanofibers were fabricated using a syringe-inside-syringe design using a NANON-03 Electrospinning set-up (MECC Co Ltd, Japan). The needle gauges used for spinning the shell and core were 18G and 27 G, respectively. The flow rate for the shell solution was 2 ml/hour and 1ml/hour for the core solution. The voltage used was 18KV with the Electrospinning distance set at 10 cm. The collector drum was rotated at about 50 rpm.

Fig. 15 depicts a phase contrast and fluorescence image of the core-shell nanofibers obtained using glycerol and Alexa Fluor-Concavalin A as the core. The fluorescence images clearly showed that the protein solution was

successfully encapsulated within the core using this method.

Fig. 16 a & b are the confocal images at different Z
5 planes of the core-shell fiber obtained. The core solution
was mixed with fluorescent protein Concavalin A and the
shell material was non-fluorescent polymer. Hence, the
differential interference contrast microscopy (DIC) image
is overlayed with fluorescent image. The fluorescent
10 material is excited with a laser and a point-by-point
scanning is performed to generate z stacking (layer-by-
layer image).

The DIC image was obtained using the passage of
light. As such, layer-by-layer scanning was not possible.
15 Fig. 16 shows the fibrous mat collected. As the fibers
are at different Z planes, fluorescence was not observed
for all of the fibers. The fibers which are at a
particular plane of measurement will exhibit fluorescence.
The Z stacking was subjected to X and Y sectioning to
20 prove the presence of fluorescent core inside the fiber.
The presence of this feature proves that the fibers are
capable of encapsulating biological material therein.

Example 7

25

Methods

Poly(caprolactone) (PCL) polymer dissolved in
Tetrahydrofuran (THF) and Dimethyl sulphoxide (DMSO) was
electrospun using a NANON-03 Electrospinning set-up using
30 a 20KV voltage and an 18G gauge needle. The
electrospinning distance was set at 15cm and the polymer
flow rate was 0.6 ml/hour. No porogen was used in this
example.

THF and DMSO are non-miscible with one another and the boiling point of THF is substantially lower than that of DMSO causing it to evaporate first leaving pores on the surface of the PCL fiber.

5

Results

Thick fibers of PCL were formed with a 29% PCL solution. 15% and 18% PCL polymer solutions were also prepared which could not be electrospun. However, a 27% PCL polymer solution produced nanofibers with lots of beads. In addition, 28% and 29% PCL polymer solutions produced uniform nanofibers without any beads (see Fig.17) The thickness of these fibers was $>5\mu\text{m}$ which is a very good size for encapsulating bacteria or other bigger bioactive molecules within the core of the PCL fiber. From high magnification SEM pictures it was observed that most of the pores have a see-through structure which in turn is beneficial in the case of core-shell nanofibers with encapsulated biomolecules as this structure will permit the of biomolecule to interact with the external environment of the fiber through the pores.

15

20

Comparison of Results

Comparing Examples 5, 6 and 7, it is clear that Example 6 provides a porous fiber that is superior in many aspects to that obtained in Example 5.

25

The utilization of PVDF as the main core polymer in the formation of the porous fiber is one of the key features that is distinctive from Example 5, which utilizes PCL as its core polymer.

30

PVDF is mechanically strong and as it is non-biodegradable it is ideal candidate for applications such as waste water treatment.

In comparison, PCL is a biodegradable polymer which has a significantly lower mechanical strength than PVDF. PCL has been shown to be degraded by the action of aerobic and anaerobic microorganisms that are widely distributed in various ecosystems (Tokiwa, 2009). As such, it can be easily degraded by lipases and esterases and hence is not suitable for use with microorganisms such as bacteria or viruses.

In addition, the increased thickness of the porous fibers ($>2\mu\text{m}$) as achieved in Example 6 is advantageous when encapsulating bacteria within its core, given that the size of bacteria is only about few microns ($0.2\text{--}2\mu\text{m}$) in width.

Further, the porous fiber of Example 6 also provides uniform porous structures that improve its efficiency, when compared to the porous fibers obtained in Examples 5 and 7. In particular, the pores present in the fiber of Example 6 act as channels to permit the interaction of the bacteria/bioorganism with the external environment of the fiber, whilst retaining the bacteria/bioorganism within the core of the fiber.

As such, the characteristics of the porous fiber of Example 6 make it suitable for use in applications such as water treatment and biosensor technologies.

Encapsulation of immobilized Enzymes and Proteins

Various types of particles have been used to study antigen-antibodies, biotin-avidin, peptides, DNA/protein detections and cellular analysis. The most common particles are gold nanoparticles, silica nanoparticles (Knopp et al, 2009), polystyrene beads (Lateef et. al. 2005) and the like. There are different approaches that can be used to prepare these micro/nanoparticles in order to modify their surface with ligands using electrostatic

interactions or physical adsorption immobilization. However, to produce more stable linkages with biomolecules, covalent attachment is preferred where the surface is chemically functionalized to produce reactive
5 groups such as -NH₂, -COOH, -SH, -CHO, -OH, peptide, carbamate linkages and the like which can covalently bind to biomolecules (see Fig. 18).

Nanoparticles functionalized with groups that provide affinity sites for the binding of biomolecules have been
10 used for the specific attachment of proteins and oligonucleotides (Li-Na 2010). Gestwicki, (2000) has used streptavidin-functionalized AuNPs for the affinity binding of biotinylated proteins or biotinylated oligonucleotides whereas Sergeev (2003) have used protein A conjugate bound
15 to AuNPs as a versatile linker to Fc fragments of various immunoglobulins and carbohydrate-modified AuNPs to recognize their respective binding proteins.

Such immobilized microparticles and nanoparticles can
20 be then encapsulated within the core of nanofibers in accordance with the disclosure using core-shell electrospinning disclosed herein. Leaching of biomolecules can be drastically reduced as these particles can be easily retained within the core of the porous fibers
25 described herein. The entrapment of biomolecules alone is not possible without immobilization to these particles within the core as the size of these biomolecules is in the order of few nanometers and the pores on the nanofibers are at least 100nm. Various combinations of
30 different sizes of particles and pores on nanofiber surface can be produced in order to retain the particles within core and increase the efficiency of the membranes (see Fig. 19).

Applications

Advantageously, the disclosed nanofiber or microfiber can be used to form a membrane in a bioreactor. Due to the pores present in the nanofiber or microfiber, a greater contact surface area can be provided between the encapsulated biological material and a target substrate, such as a contaminant, in an external environment. Due to the greater contact area, the biological material can interact with the target substrate with higher efficiency, leading to a decrease in the reaction time between the biological material and the substrate.

Advantageously, as the encapsulated biological material is already present in the bioreactor, the start-up time associated with the growth of the biological material is reduced as compared to a conventional bioreactor.

Advantageously, since the target substrate is the food source for the biological material, the occurrence and extent of membrane fouling in which the substrate builds up on the surface of the nanofiber or microfiber are substantially reduced as compared to a conventional bioreactor. The pores present in the nanofiber or microfiber allow the biological material to interact directly with the target substrate, leading to the removal of the target substrate. Since the target substrate is removed by the action of the biological material, the build-up of such target substrate is substantially reduced. Accordingly, the problems of build-up and the subsequent membrane fouling are substantially reduced in the present bioreactor.

Advantageously, the disclosed membrane with biological material encapsulated within can be used for membrane filtration processes.

The membrane bioreactor or membrane contactor may be used in bioremediation, bioprocess and in the production of foodstuffs and pharmaceuticals.

Advantageously, the disclosed fiber encapsulating a biological material therein can be used in industries such as fermentation, catalysis of chemical reactions, food processing, paper industries, biofuel industries, waste treatment and medical industries.

Advantageously, the disclosed nanofiber or microfiber can be used as a biosensor in which the biological material such as protein, cell, enzyme or nucleic acids can be used to detect changes in an environment system or sample.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Reference

(1) James B. Sumner, Charles V. Noback. The estimation of sugar in diabetic urine using dinitrosalicylic acid. The Journal of Biological Chemistry, (1924) Vol. LXII No 2.

(2) Ying Yang, Andrea Centrone, Liang Chen, Fritz Simeon, T. Alan Hatton, Gregory C. Rutledge, Carbon, (2011) 49 3395 -3403.

(3) Dietmar Knopp, Dianping Tang, Reinhard Niessner, Analytica Chimica Acta 647 (2009) 14-30).

(4) Syed S. Lateef, Samuel Boateng, Neil Ahluwalia, Thomas J. Hartman, Brenda Russell, Luke Hanley, J Biomed Mater Res A. (2005); 72(4):373-80.

(5) Ma Li-Na, LIU Dian-Jun, WANG Zhen-Xin, Chinese J. Sof Anal. Chem. (2010) Vol.38:(1) 1-7.

(6) Gestwicki J E, Strong L E, Kisseling L L. Angew. Chem. Int. Ed., (2000) 39 (24): 4567-4570

(7) Sergeev B M, Kiryukhin M V, Rubtsova M Y, Prusov A N. Colloid J., (2003) 65 (5): 636-638

10Y (8) Yutaka Tokiwa, Buenaventurada P. Calabria, Charles U. Ugw and Seiichi Aiba, Int. J. Mol. Sci. (2009) 10, 3722-3742; doi:10.3390/ijms10093722

Claims

1. A porous fiber having a core-shell configuration, said fiber comprised of a non-
5 biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material therein.

2. A porous fiber as claimed in claim 1, wherein
10 the fiber has a generally longitudinal shape.

3. A porous fiber as claimed in claim 1 or claim 2, wherein said biological material is dispersed throughout the length of the longitudinal fiber.
15

4. The porous fiber of any one of claims 1 to 3, wherein the porosity of the fibers is in the range of 5 % to 90 %.

20 5. The porous fiber of any one of claims 1 to 4, wherein the pores of said fiber have a pore size in the range of 1 nm to 1000 nm.

25 6. The porous fiber of any one of claims 1 to 5, wherein said biological material is selected from the group consisting of bacteria, viruses, algae, fungi, cells and yeast.

30 7. The porous fiber of any one of claims 1 to 5, wherein said biological material is selected from the group consisting of enzymes, proteins and nucleic acids.

8. The porous fiber as claimed in claim 7, wherein the biological material is immobilized on a solid substrate.

5 9. The porous fiber of any one of claims 1 to 8, wherein said fiber is an electrospun fiber.

10 10. The porous fiber of any one of claims 1 to 9, wherein the non-biodegradable polymer is polyvinylidene fluoride.

11. The porous fiber as claimed in any one of claims 1 to 10, wherein the polymer has a tensile strength of at least 20 MPa.

15 12. The porous fiber as claimed in claim 11, wherein the polymer has a tensile strength of at least 50 MPa.

20 13. A porous fiber as claimed in any one of claims 1 to 10, wherein the polymer has a tensile modulus of at least 400 MPa.

25 14. A porous fiber as claimed in claim 13, wherein the polymer has a tensile modulus of at least 1700 MPa.

15. The porous fiber of any one of claims 1 to 14, wherein said fiber is a nanofiber.

30 16. The porous fiber of any one of claims 1 to 14, wherein said fiber is a microfiber.

17. The porous fiber of any one of claims 1 to 14, wherein said biological material is a particle having a

particle size in the nano-sized range or micron-sized range.

18. The porous fiber of claim 1, wherein said
5 biological material is selected to have a bioremediation activity.

19. A method of forming a porous fiber having a
core-shell configuration, said fiber comprised of a non-
10 biodegradable polymer, wherein the pores on the fiber are
configured to encapsulate and thereby retain a biological
material comprising the steps of:

- a. providing a core solution of the biological material;
- 15 b. providing a mixture comprising a fiber material comprised of the non-biodegradable polymer and a pore-forming material as the shell solution, wherein the fiber material and the pore forming material are miscible with each other;
- 20 c. forming a core-shell fiber from said core solution and shell solution, said fiber encapsulating and retaining said biological material in the core; and
- d. removing said pore-forming material from said
formed core-shell fiber to create pores therein.

25

20. The method of claim 19, wherein said forming step comprises the step of electrospinning said core solution and shell solution onto a collector.

30

21. The method of claim 19, wherein said pore-forming material is a solvent having a boiling point in the range of 30 °C to 200 °C.

22. The method of claim 21, wherein said removing step comprises the step of evaporating said solvent from said core-shell fiber to form said porous fiber.

5 23. The method of claim 21, wherein said solvent comprises an organic solvent.

24. The method of claim 19, wherein said pore-forming material is a polymer that is soluble in a
10 solvent.

25. The method of claim 20, wherein said removing step comprises the step of dissolving said soluble polymer in a solvent such that pores are formed in the
15 core-shell fiber.

26. Use of a porous fiber having a core-shell configuration, said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are
20 configured to encapsulate and thereby retain a biological material therein, for the removal of contaminants from an environmental sample.

27. Use of a porous fiber having a core-shell
25 configuration, said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material therein, as a biosensor.

30 28. A filtration membrane comprising a plurality of porous fibers, each porous fiber having a core-shell configuration, said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are

configured to encapsulate and thereby retain a biological material therein.

29. A bioreactor comprising a membrane module,
5 wherein said membrane module comprises a plurality of porous fibers, each porous fiber having a core-shell configuration, said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological
10 material therein.

30. A membrane contactor comprising a membrane module, wherein said membrane module comprises a plurality of porous fibers, each porous fiber having a core-shell
15 configuration, said fiber comprised of a non-biodegradable polymer, wherein the pores on the fiber are configured to encapsulate and thereby retain a biological material therein.