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(54) **BACILLUS BOMBYSEPTICUS SF3
DECOMPOSING FLUORINE-CONTAINING
COMPOUND, RECOMBINANT
MICROORGANISM INCLUDING GENE
DERIVED FROM BACILLUS
BOMBYSEPTICUS AND METHOD OF
REDUCING CONCENTRATION OF
FLUORINE-CONTAINING COMPOUND IN
SAMPLE BY USING BACILLUS
BOMBYSEPTICUS**

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(57) **ABSTRACT**

Provided are a microorganism having activity in reducing a concentration of a fluorine-containing compound in a sample, a recombinant microorganism including a gene derived from the microorganism, and a method of reducing the concentration of the fluorine-containing compound in the sample by using the microorganism or recombinant microorganism.

Specification includes a Sequence Listing.

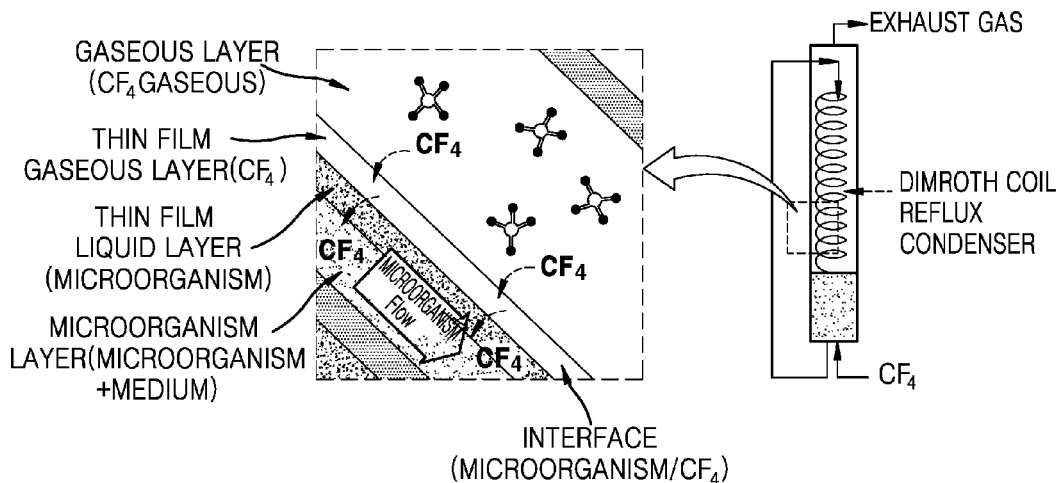
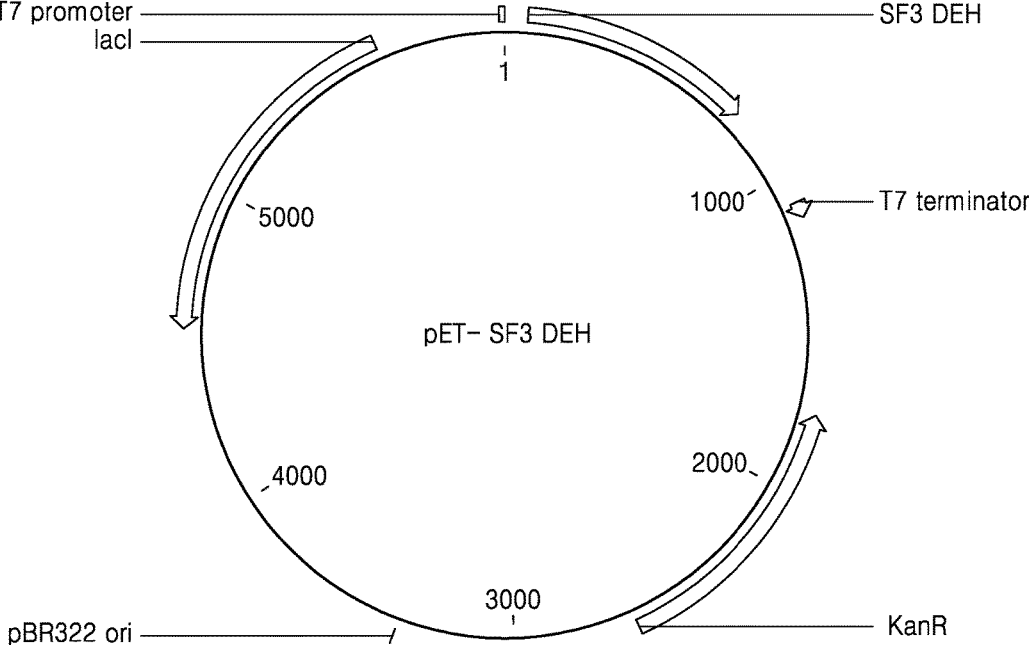


FIG. 1



*SF3 DEH: GENE_00757, GENE_01351, OR GENE_04275

FIG. 2

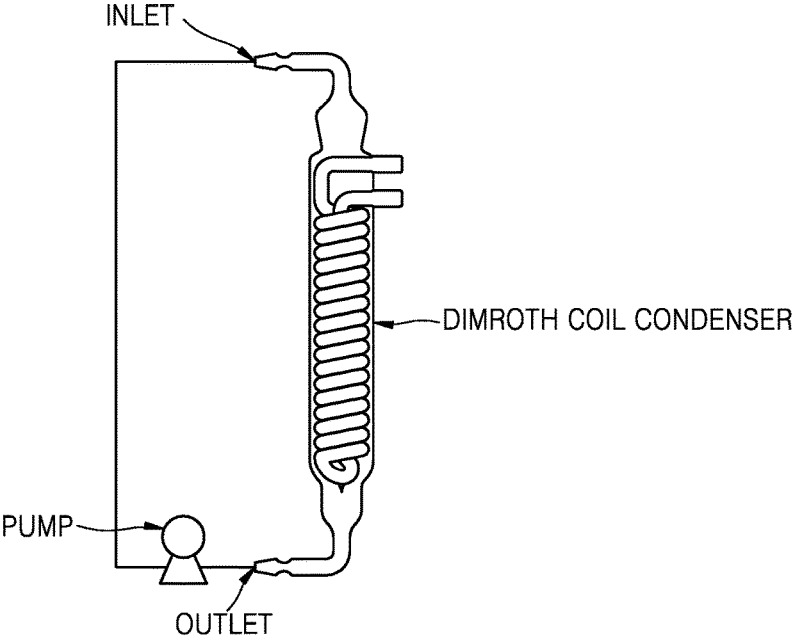


FIG. 3

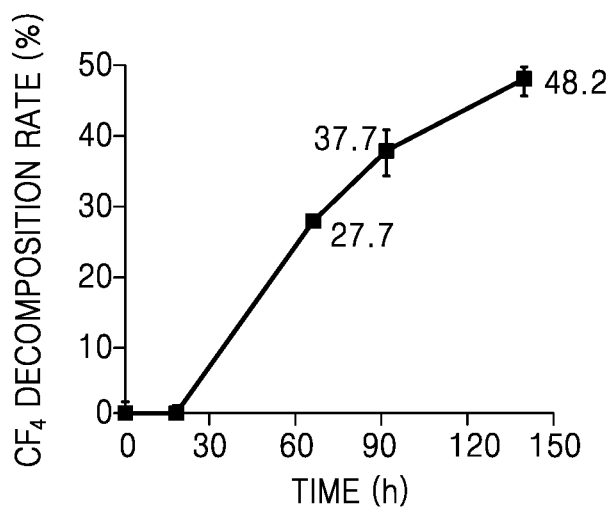


FIG. 4

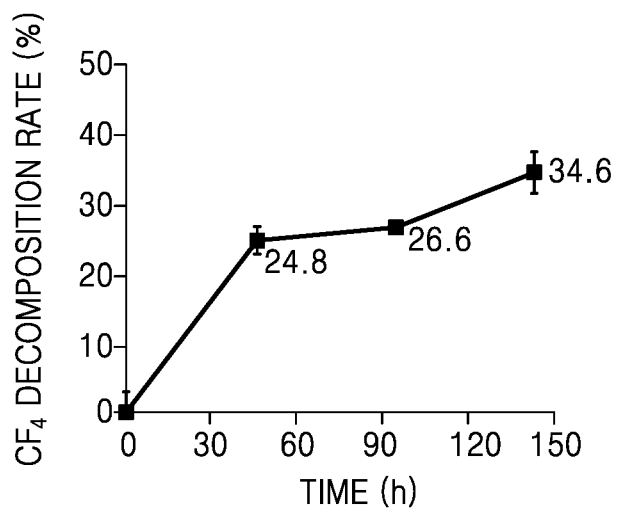


FIG. 5

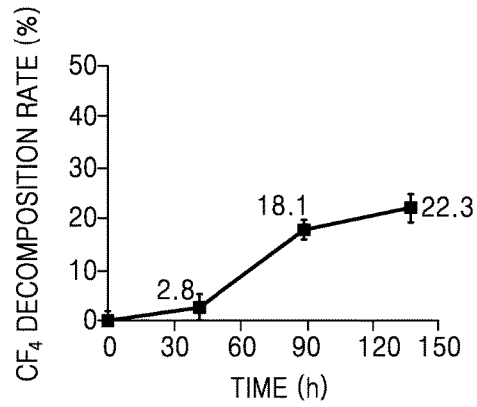
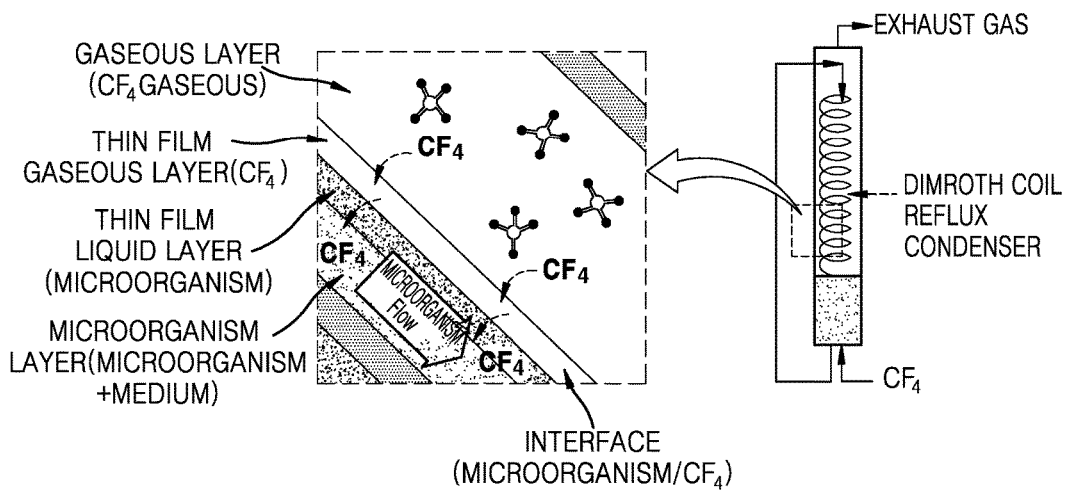


FIG. 6



**BACILLUS BOMBYSEPTICUS SF3
DECOMPOSING FLUORINE-CONTAINING
COMPOUND, RECOMBINANT
MICROORGANISM INCLUDING GENE
DERIVED FROM BACILLUS
BOMBYSEPTICUS AND METHOD OF
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SAMPLE BY USING BACILLUS
BOMBYSEPTICUS**

CROSS-REFERENCE TO RELATED
APPLICATION

[0001] This application claims the benefit of Korean Patent Application No. 10-2017-0093683, filed on Jul. 24, 2017, in the Korean Intellectual Property Office, the entire disclosure of which is hereby incorporated by reference.

INCORPORATION-BY-REFERENCE OF
MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 11,132 Byte ASCII (Text) file named “737826_ST25.TXT,” created on Jun. 7, 2018.

BACKGROUND

[0003] The emission of greenhouse gases, which have accelerated global warming, is a serious environmental problem, and regulations to reduce and prevent the emissions of greenhouse gases have been tightened. Among the greenhouse gases, fluorinated gases (F-gases), such as per-fluorocarbons (PFCs), hydrofluorocarbon (HFCs), or sulfur hexafluoride (SF₆) show low absolute emission but have a long half-life and a very high global warming potential, resulting in significantly adverse environmental impact. The amount of F-gases emitted from the semiconductor and electronics industries, which are major causes of F-gas emission, has exceeded the assigned amount of greenhouse gas emissions and continues to increase. Therefore, costs required for decomposition of greenhouse gases and greenhouse gas emission allowances are increasing every year.

[0004] A pyrolysis or catalytic thermal oxidation process has generally been used in the decomposition of F-gases. However, this process has disadvantages of limited decomposition rate, emission of secondary pollutants, and high cost. However, biological decomposition of F-gases would allow F-gases to be treated in a more economical and environmentally-friendly manner.

[0005] Therefore, there is a need to develop new microorganisms and methods for the biological decomposition of F-gases. This invention provides such microorganisms and methods.

SUMMARY

[0006] Provided herein is a microorganism referred to as *Bacillus bombysepticus* SF3 (KCTC 13220BP) having activity in reducing a concentration of a fluorine-containing compound in a sample.

[0007] Also provided is a recombinant microorganism having a genetic modification that increases the level of a

polypeptide having a sequence identity of about 90% or more with respect to an amino acid sequence of SEQ ID NO: 1, 2, or 3.

[0008] Provided is a composition for use in reducing a concentration of a fluorine-containing compound in a sample, the composition including *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism having a genetic modification that increases the level of a polypeptide having a sequence identity of about 90% or more with respect to an amino acid sequence of SEQ ID NO: 1, 2, or 3.

[0009] Provided is a method of reducing a concentration of a fluorine-containing compound in a sample, the method including contacting a sample including a fluorine-containing compound with *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism having a genetic modification that increases the level of a polypeptide having a sequence identity of about 90% or more with respect to an amino acid sequence of SEQ ID NO: 1, 2, or 3, so as to reduce the concentration of the fluorine-containing compound in the sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] These and/or other aspects will become apparent and more readily appreciated from the following description of the embodiments, taken in conjunction with the accompanying drawings in which:

[0011] FIG. 1 is a vector map of a pET-SF3 DEH vector;

[0012] FIG. 2 is a schematic diagram of a reactor used in Example 3;

[0013] FIG. 3 is a graph showing decomposition rates of CF₄ when a strain of *B. bombysepticus* SF3 is brought into contact with a fluorine-containing compound;

[0014] FIG. 4 is a graph showing decomposition rates of CF₄ when a strain of BL21/pET-SF3 00757 is brought into contact with a fluorine-containing compound;

[0015] FIG. 5 is a graph showing decomposition rates of CF₄ when a strain of *Bacillus cereus* is brought into contact with a fluorine-containing compound; and

[0016] FIG. 6 is a schematic diagram for decomposing CF₄ by applying a gas-phase circulation process using a microorganism.

DETAILED DESCRIPTION

[0017] Reference will now be made in detail to embodiments, examples of which are illustrated in the accompanying drawings, wherein like reference numerals refer to like elements throughout. In this regard, the present embodiments may have different forms and should not be construed as being limited to the descriptions set forth herein. Accordingly, the embodiments are merely described below, by referring to the figures, to explain aspects. As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items. Expressions such as “at least one of,” when preceding a list of elements, modify the entire list of elements and do not modify the individual elements of the list.

[0018] The term “increase in the level of a polypeptide” as used herein may refer to a detectable increase in the amount or concentration of a polypeptide in a cell. The term “increase in the level of a polypeptide” may refer to a level of a polypeptide in a cell, such as a genetically modified cell, that is higher than the level of the polypeptide in a com-

parative cell of the same type, such as a cell that does not have a given genetic modification. Any increase of any amount is encompassed. The increase in the level of a polypeptide of a given cell (e.g., a cell with a given genetic modification) may be, for instance, about 5% or greater, about 10% or greater, about 15% or greater, about 20% or greater, about 30% or greater, about 50% or greater, about 60% or greater, about 70% or greater, or about 100% or greater, than a comparative cell (e.g., a cell of the same type without the genetic modification).

[0019] The increase in the level of a polypeptide may be achieved by an increase in expression of a gene encoding the polypeptide. The increase in the expression may be achieved by introduction of a polynucleotide encoding the polypeptide to a cell; an increase in the copy number of the gene encoding the polypeptide, or a modification on a regulatory region of the polynucleotide encoding the polypeptide that increases expression of the polynucleotide. The polynucleotide encoding the polypeptide may be operably linked to a regulatory sequence that allows expression thereof, for example, a promoter, an enhancer, a polyadenylation region, or a combination thereof. The polynucleotide which is introduced into the cell or whose copy number is increased in the cell may be endogenous or heterologous to the cell. The term “endogenous gene” refers to a gene which is included in a microorganism prior to introducing the genetic modification (e.g., a native gene). The term “heterologous” refers to a gene that is “foreign,” or “not native” to the species. In either case, a polynucleotide or gene that is introduced into a cell is referred to as “exogenous,” and an exogenous gene or polynucleotide may be endogenous or heterologous with respect to a cell into which the gene is introduced. Thus, the microorganism into which the polynucleotide encoding the polypeptide is introduced may be a microorganism that already includes the gene encoded by the polynucleotide (e.g., the gene or polynucleotide is endogenous to the microorganism). Alternatively, the microorganism can be without a copy of the gene prior to its introduction (e.g., the polynucleotide or gene is heterologous to the microorganism).

[0020] The term “increase of copy number” as used herein may be caused by introduction of an exogenous polynucleotide or amplification of an endogenous gene. In an embodiment, the increase of copy number may be caused by a genetic modification such as introduction of a gene that does not exist in a non-engineered microorganism. In other words, the recombinant microorganism can be engineered to comprise additional copies of an endogenous gene, or can comprise one or more copies of a heterologous gene. The introduction of such a gene may be mediated by a vehicle such as a vector. The introduction may be achieved by transient introduction in which the gene is not integrated into a genome, or by insertion of the gene into the genome. The introduction may be achieved by, for example, introducing a vector into the cell, and then replicating the vector in the cell, wherein the vector includes a polynucleotide encoding a target polypeptide, or by integrating the polynucleotide into the genome.

[0021] The introduction of the gene may be performed by any known method in the art, such as transformation, transfection, or electroporation. The gene may be introduced via a vehicle or may be introduced by itself. The term “vehicle” as used herein may refer to a nucleic acid molecule that is able to deliver other nucleic acids linked thereto. As

a nucleic acid sequence mediating introduction of a specific gene, the vehicle as used herein may be construed to be interchangeable with a vector, a nucleic acid structure, and a cassette. The vector may include, for example, a plasmid vector or a virus-derived vector. The plasmid may include a circular double-stranded DNA ring linkable with another DNA. The vector may include, for example, a plasmid expression vector, a virus expression vector, such as a replication-defective retrovirus, adenovirus, and adeno-associated virus, or a combination thereof.

[0022] The term “parent cell” as used herein refers to a cell lacking a particular genetic modification. The parent cell can be an original cell, for example, a non-genetically modified cell of the same type as the genetically engineered microorganism. Also, the “parent cell” may be a cell that lacks a particular genetic modification, but is identical in all other respects. Thus, the parent cell may be a cell that is used as a starting material to produce a genetically engineered microorganism having increased activity of a given protein (for example, a protein having a sequence identity of about 90% or more to a dehalogenase). The same comparison may be also applied to other genetic modifications.

[0023] The term “gene” as used herein may refer to a polynucleotide expressing a specific protein. A gene may include regulatory sequences, such as a 5' non-coding sequence and/or a 3' non-coding sequence, or may be free from regulatory sequences.

[0024] The term “sequence identity” of a nucleic acid or polypeptide as used herein refers to a degree of identity between nucleotides or amino acid residues of sequences obtained after the sequences are aligned so as to best match in certain comparable regions. The sequence identity is a value measured by comparing two sequences in certain comparable regions via optimal alignment of the two sequences, in which portions of the sequences in the certain comparable regions may be added or deleted compared to reference sequences. A percentage of sequence identity may be calculated by, for example, comparing two optimally aligned sequences in the entire comparable regions, determining the number of locations in which the same amino acids or nucleic acids appear to obtain the number of matching locations, dividing the number of matching locations by the total number of locations in the comparable regions (that is, the size of a range), and multiplying a result of the division by 100 to obtain the percentage of the sequence identity. The percentage of the sequence identity may be determined using a known sequence comparison program, for example, BLASTN (NCBI), BLASTP (NCBI), CLC Main Workbench (CLC bio), or MegAlign™ (DNASTAR Inc).

[0025] The term “genetic modification” as used herein may refer to an artificial modification in a constitution or structure of a genetic material of a cell.

[0026] The symbol “%” as used herein indicates w/w %, unless otherwise stated.

[0027] An aspect of the present invention provides a polypeptide having a sequence identity of about 90% or more with respect to an amino acid sequence of SEQ ID NO: 1, 2, or 3.

[0028] The polypeptide may include a detectable label attached thereto. The detectable label may be a fluorescent material, or a material having a specific binding ability, or a material capable of binding to the material having a specific binding ability.

[0029] The polypeptide may be dehalogenase. The term “dehalogenase” as used herein may refer to an enzyme that catalyzes the removal of a halogen atom from a substrate. The dehalogenase may be 4-chlorobenzoate dehalogenase, 4-chlorobenzoyl-CoA dehalogenase, dichloromethane dehalogenase, fluoroacetate dehalogenase, haloacetate dehalogenase, (R)-2-haloacid dehalogenase, (S)-2-haloacid dehalogenase, haloalkane dehalogenase, halohydrin dehalogenase, or tetrachloroethene reductive dehalogenase. For example, the dehalogenase may belong to a haloacid dehalogenase superfamily. The haloacid dehalogenase superfamily may be EC 3.8.1.2. However, the present disclosure should not be construed as being limited to this particular mechanism. The polypeptide may have a sequence identity of about 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more, with respect to an amino acid sequence of SEQ ID NOs: 1, 2, or 3. The polypeptide may include an amino acid sequence selected from SEQ ID NOs: 1, 2, or 3.

[0030] Another aspect of the invention provides a polynucleotide including a nucleotide sequence encoding a polypeptide having a sequence identity of about 90% or more with respect to an amino acid sequence of SEQ ID NO: 1, 2, or 3. In an embodiment, the polynucleotide sequence comprises SEQ ID NO: 4, 5, or 6.

[0031] The polynucleotide may be in a vector. The vector may be an expression vector, which is configured to express a foreign gene inserted into the vector in a host organism. The vector may include an origin, a promoter, a cloning site, a marker, or a combination thereof. The vector may be, for example, a plasmid. The polynucleotide may be inserted into a cloning site in association with an open reading frame, so as to be expressed in a host organism. In one embodiment, the vector includes a promoter operably linked to a nucleic acid sequence comprising SEQ ID NO: 4, 5, or 6.

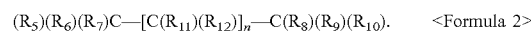
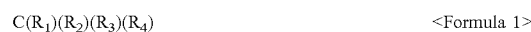
[0032] Another aspect of the invention provides a recombinant microorganism including a genetic modification that increases a level of a polypeptide or a combination thereof, the polypeptide having a sequence identity of about 90% or more with respect to an amino acid sequence of SEQ ID NO: 1, 2, or 3.

[0033] The genetic modification may include an increase in the copy number of a gene encoding the polypeptide. The genetic modification may include introduction of an exogenous polynucleotide encoding the polypeptide, such as by transformation, transfection, or electroporation of the polynucleotide encoding the polypeptide. The recombinant microorganism may be a microorganism to which the gene encoding the polypeptide is introduced. The gene may have a sequence identity of 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more, with respect to a nucleotide sequence of SEQ ID NO: 4, 5, or 6. The recombinant microorganism may belong to the genus *Escherichia*, *Bacillus*, *Pseudomonas*, *Xanthobacter*, or *Saccharomyces*. In an embodiment, the recombinant microorganism may be *E. coli* or *B. bombysepticus*.

[0034] Another aspect of the invention provides a method for preparing the inventive recombinant microorganisms described herein, the method comprising introducing into a microorganism a genetic modification that increases the level of a polypeptide comprising the amino acid sequence of SEQ ID NO: 1, 2, or 3. In an embodiment the method comprises introducing into the microorganism an exogenous, optionally heterologous, nucleic acid that encodes the

polypeptide. In an embodiment the exogenous, optionally heterologous, nucleic acid comprises SEQ ID NO: 4, 5, or 6 or has a sequence identity of 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more thereto. In certain embodiments the microorganism for the method for preparing the inventive microorganism is selected from the genus *Escherichia*, *Bacillus*, *Pseudomonas*, *Xanthobacter*, or *Saccharomyces*.

[0035] The recombinant microorganism may have activity in reducing a concentration of a “fluorine-containing compound” in a sample. The “fluorine-containing compound” may be an alkane compound having 1 to 12 carbon atoms substituted with at least one fluorine. The term “fluorine-containing compound” as used herein may be represented by Formula 1 or Formula 2:



[0036] In Formula 1 and 2, n is be an integer from 0 to 10, and when n is equal or greater than 2, each R_{11} can be the same or different, and each of R_{12} can be the same or different,

[0037] R_1 , R_2 , R_3 , and R_4 are each independently fluorine (F), chlorine (Cl), bromine (Br), iodine (I), or hydrogen (H), wherein at least one selected from R_1 , R_2 , R_3 , and R_4 is F, and

[0038] R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} are each independently F, Cl, Br, I, or H, wherein at least one selected from R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} is F.

[0039] In an embodiment, in Formula 1 and 2, n may be an integer from 0 to 3, an integer from 0 to 4, an integer from 0 to 5, or an integer from 0 to 7.

[0040] In another embodiment, the fluorine-containing compound may be CH_3F , CH_2F_2 , CHF_3 , CF_4 , or a mixture thereof.

[0041] Another aspect of the invention provides a composition for use in reducing a concentration of a fluorine-containing compound in a sample, the composition including *B. bombysepticus* SF3 (KCTC 13220BP) or any recombinant microorganism described herein. In certain embodiments the composition may comprise a fluorine-containing compound, such as those described herein.

[0042] The recombinant microorganism is the same as described above. Without wishing to be bound by any particular mechanism of action, it is believed the composition reduces the concentration of a fluorine-containing compound in the sample by cleaving a C—F bond of the fluorine-containing compound; converting the fluorine-containing compound into a different substance; or accumulating the fluorine-containing compound in a cell.

[0043] The sample may be a liquid sample, a gaseous sample, or a combination thereof. The sample may be free of the recombinant microorganism. The sample may be industrial sewage or waste gas. For example, the sample may be industrial sludge. The term “sludge” refers to a semi-solid slurry and can be produced as sewage sludge from wastewater treatment processes or as a settled suspension obtained from conventional drinking water treatment and numerous other industrial processes.

[0044] In the composition, the *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism may be contained in a reactor, wherein the reactor comprises a vessel for holding or flowing the *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism, a

sample, or the combination thereof, comprising inlet and outlet for the *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism, a sample, or the combination thereof.

[0045] Another aspect of the invention provides a method of reducing a concentration of a fluorine-containing compound in a sample, the method including contacting a sample including a fluorine-containing compound with *Bacillus bombysepticus* SF3 (KCTC 13220BP) or a recombinant microorganism described herein (e.g., comprising a genetic modification that increases the level of a polypeptide having a sequence identity of about 90% or more with respect to the amino acid sequence of SEQ ID NO: 1, 2, or 3), so as to reduce the concentration of the fluorine-containing compound in the sample.

[0046] The sample may be a liquid sample, a gaseous sample, or a combination thereof. In some embodiments, the sample is substantially or completely free of a recombinant microorganism as described herein prior to contacting the sample with the recombinant microorganism. The sample may be industrial waste water or waste gas. For example, the sample may be industrial sludge.

[0047] Contacting the sample with the microorganism may be performed while the sample is in a liquid phase, a gaseous phase, or a combination thereof. The contacting may include culturing the *B. bombysepticus* (e.g., KCTC 13220BP), the recombinant microorganism, or a combination thereof, in the presence of the fluorine-containing compound or a sample comprising the same. The contacting may be performed in an air-tight sealed container. The contacting may be performed when the growth stage of the *B. bombysepticus* (e.g., KCTC 13220BP) or the recombinant microorganism is in an exponential phase or a stationary phase. The culturing may be performed under aerobic or anaerobic conditions. The contacting may be performed under conditions where the *B. bombysepticus* (e.g., KCTC 13220BP), the recombinant microorganism, or a combination thereof may survive in the closed container. Such conditions appropriate for the survival of the *B. bombysepticus* (e.g., KCTC 13220BP), the recombinant microorganism, or a combination thereof may include conditions where the *B. bombysepticus* (e.g., KCTC 13220BP), the recombinant microorganism, or a combination thereof may proliferate or may be allowed to be in a resting state.

[0048] The contacting may include passive contacting and/or active contacting. The term 'passive contacting' refers to a contacting without an external driving force and the term 'active contacting' refers to a contacting with an external driving force. The contacting may be achieved in a way that the fluorine-containing compound is injected in the form of bubbles into a solution containing the *B. bombysepticus* (e.g., KCTC 13220BP) and/or the recombinant microorganism, or is sprayed. For example, the contacting may be achieved by blowing the sample into a medium or a culture broth. By way of further illustration, for the injection of the sample, the sample may be blown from the bottom of the medium or the culture broth to the top thereof. The injection of the sample may be achieved by making droplets of the sample. The contacting may be performed in a batch or continuous manner. The contacting may be performed repeatedly, such as two or more times, for example, three times, five times, or ten times or more. The contacting may be continued or repeated until the fluorine-containing compound is reduced to a desired concentration.

[0049] In the method, the contacting may be performed in a reactor *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism is contained in a vessel, wherein the reactor comprises a vessel for holding or flowing the *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism, a sample, or the combination thereof, comprising one or more inlets and outlets for the *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism, a sample, or the combination thereof.

[0050] In some embodiments, the *B. bombysepticus* (e.g., KCTC 13220BP), the recombinant microorganism, or a combination thereof may be in the form of a thin film layer, such as a liquid thin film layer. The fluorine-containing compound or sample comprising the same may be in the form of a gaseous thin film layer. The liquid thin film layer formed by the *B. bombysepticus* (e.g., KCTC 13220BP), the recombinant microorganism, or a combination thereof and the gaseous thin film layer formed by the fluorine-containing compound may contact each other according to the method.

[0051] In an embodiment, the method can comprise subjecting the *B. bombysepticus* (e.g., KCTC 13220BP), the recombinant microorganism, or a combination thereof to a circulation process, so that the contact area or the time of contact of the microorganism with the fluorine-containing compound, or sample comprising the same, may increase. The circulation process may increase the mass transfer coefficient (KLa) value, as well as increase the amount and/or rate of decomposition of the fluorine-containing compound.

[0052] The contacting of the inventive method may further include using an exhaust gas decomposition device including one or more reactors each of which includes at least a first inlet and a first outlet. Such a method can involve:

[0053] (a) injecting the sample into the exhaust gas decomposition device and

[0054] (b) injecting *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism into the device through the at least one first inlet (the microorganism and sample can, for instance, be introduced through the same inlet or different inlets), so that *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism may contact the sample and the resulting mixture may be discharged through the first outlet.

[0055] In some embodiments, the exhaust gas decomposition device may include a second inlet and a second outlet, and the sample may be injected through the second inlet and discharged through the second outlet. In such a configuration, the *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism can move in a direction opposite to a direction in which the sample moves, for instance, by supplying the microorganism through a different inlet and discharging from a different outlet than the sample. In still other embodiments, a fluid thin film including *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism may be formed on an inner wall of the one or more reactors.

[0056] The exhaust gas decomposition device used in the method may further include a first circulation line for re-supplying at least a portion of a fluid to the at least one first inlet, wherein the fluid contains *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism discharged through the first outlet. The sample including the fluorine-containing compound may remain inside the one or more reactors, or may be circulated. In addition, the one or

more reactors of the exhaust gas decomposition device may further include a second inlet and a second outlet, wherein the sample may be supplied into the one or more reactors through the second inlet and discharged to the outside of the one or more reactors through the second outlet. The sample may, then, move along a second direction within the one or more reactors, wherein the second direction may be different from (e.g., generally opposite) the direction in which *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism moves. In addition, in at least one of a fluid collection zone at the bottom of the inside of the one or more reactors and a fluid reaction zone at the top of the inside of the one or more reactors of the exhaust gas decomposition device, the fluid containing *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism and the sample including the fluorine-containing compound may contact each other, thereby decomposing the fluorine-containing compound. In the fluid reaction zone, a fluid thin film including the fluid containing *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism may contact a fluid including the sample.

[0057] The exhaust gas decomposition device used in the method may further include a structure inside the one or more reactors, wherein the structure may be configured to increase the contact area between the fluid including *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism and the sample including the fluorine-containing compound. Any structure configured to increase a contact area between the fluid including *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism and the sample including the fluorine-containing compound may be included. For example, the structure may comprise a packing or a reflux tube, but is not limited thereto. The 'packing material' may be inert solid material. The packing material may be of various shapes. The packing material may be the same material used in the packing of a packed bed tower. The packing material may be made of plastic, magnetic material, steel or aluminum. The packing material may have very thin thickness. The packing material may have a ring shape such as rashing ring, pall ring, and berl saddle, a saddle type, and protrusion type. The packing material may be irregularly packed in the packed bed reactor. The packing material may efficiently increase contact between the fluorine-containing compound with a microorganism present in a liquid. The time or opportunity for contact between the fluorine-containing compound and a microorganism can be maximized by forming a thin film of a microorganisms on the surface of the packing material as well as on the inner surface of the reactor. In addition, the at least one first inlet may be connected to the fluid reaction zone at the top of the inside of the one or more reactors in the exhaust gas decomposition device, to thereby supply the fluid including *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism through the at least one first inlet.

[0058] According to an aspect of the method, the fluid including *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism may be collected in the fluid collection zone at the bottom of the inside of the one or more reactors in the exhaust gas decomposition device. The sample including the fluorine-containing compound supplied into the one or more reactors through the second inlet may pass through, in the form of bubbles, the collected fluid including *B. bombysepticus* SF3 (KCTC 13220BP) or the

recombinant microorganism to be transferred to the fluid reaction zone at the top of the inside of the one or more reactors, and then, may be discharged to the outside of the one or more reactors through the second outlet.

[0059] In the exhaust gas decomposition device, the aspect ratio of a height H of the one or more reactors to the diameter D of the one or more reactors (H/D) may be 2 or more, 5 or more, 10 or more, 15 or more, 20 or more, or 50 or more.

[0060] Furthermore, the exhaust gas decomposition device may be arranged in a way that the side-wall of one or more reactors, or some other internal surface thereof, is tilted or inclined at an angle of less than or greater than 90° relative to the surface of the earth. For example, the side-wall or other internal surface thereof can be tilted or inclined in a range of about 30° to about 150° (e.g., about 30° to less than 90° or greater than 90° to about 150°), about 70° to about 110° (e.g., about 70° to less than 90° or greater than 90° to about 110°), about 80° to about 100° (e.g., about 80° to less than 90° or greater than 90° to about 100°), or about 50° to about 90° (e.g., about 50° to less than 90°), with respect to the surface of the earth.

[0061] Regarding the method, the one or more reactors in the exhaust gas decomposition device may rotate. The fluid containing *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism may be liquid, and the sample including the fluorine-containing compound may be gas.

[0062] Hereinafter, the present invention will be described in more detail with reference to Examples. However, these Examples are provided for illustrative purposes only, and the invention is not intended to be limited by these Examples.

Example 1: Selection of Strain of *Bacillus bombysepticus* SF3 and Decomposition of Fluorine-Containing Compound Using the Strain

[0063] In Example 1, a microorganism capable of reducing a concentration of CF₄ in waste water of a semiconductor factory was selected.

[0064] Sludge in waste water discharged from Samsung Electronics Plant (Giheung, Korea) was smeared on an agar plate including a carbon-free medium (supplemented with 0.7 g/L of K₂HPO₄, 0.7 g/L of MgSO₄·7H₂O, 0.5 g/L of (NH₄)₂SO₄, 0.5 g/L of NaNO₃, 0.005 g/L of NaCl, 0.002 g/L of FeSO₄·7H₂O, 0.002 g/L of ZnSO₄·7H₂O, 0.001 g/L of MnSO₄, and 15 g/L of Agar), and the agar plate was put in a GasPak™ Jar (BD Medical Technology). The inside of the jar was filled with 99.9 v/v % of CF₄, and the jar was sealed for the standing culture at a temperature of 30□ under anaerobic conditions. Single colonies formed on the agar plate after the culture were cultured using a high throughput screening (HTS) system (Thermo Scientific/Liconic/Perkin Elmer). Each of the cultured single colonies were then inoculated on a 96-well microplate in which each well contained 100 μL of an LB medium. The 96-well microplate was subjected to the standing culture at a temperature of about 30□ for 96 hours under aerobic conditions. Meanwhile, the growth ability of the single colonies was observed by measuring the absorbance thereof at 600 nm every 12 hours. The LB medium used herein included 10 g/L of tryptone, 5 g/L of yeast extract, and 10 g/L of NaCl.

[0065] The top 2% of strains showing excellent growth ability were selected, and then inoculated in a glass serum bottle (volume of 75 mL) containing 10 mL of an LB medium to have OD₆₀₀ of 0.5. The glass serum bottle was sealed, and CF₄ was injected thereto using a syringe to have

1,000 ppm of CF₄ gas. The glass serum bottle was incubated in a shaking incubator for 4 days at a temperature of 30°C while being stirred at a speed of 230 rpm. Then, an amount of CF₄ in a headspace of the glass serum bottle was analyzed.

[0066] For the analysis, 0.5 ml of the headspace gas in the glass serum bottle was collected using a syringe and injected into gas chromatography (GC, Agilent 7890, Palo Alto, Calif., USA). The injected headspace sample was separated through a CP-PoraBOND Q column (25 m length, 0.32 mm i.d., 5 μm film thickness, Agilent), and changes in the CF₄ concentration were analyzed by a Mass Selective Detector (MSD) (Agilent 5973, Palo Alto, Calif., USA). Helium was used as carrier gas, and applied to the column at a flow rate of 1.5 ml/min in the gas chromatography column. GC conditions were as follows: an inlet temperature was 250°C; and an initial temperature was maintained at 40°C for 2 minutes and raised to 290°C at a rate of 20°C/min. Mass spectrometry (MS) conditions were as follows: an ionization energy was 70 eV, an interface temperature was 280°C, an ion source temperature was 230°C, and a quadrupole temperature was 150°C. As a control group, the headspace sample having the CF₄ concentration of 1,000 ppm was incubated in the same manner in a glass serum bottle containing no cells, followed by being subjected to the measurement.

[0067] Consequently, compared to the control group having no cells, the concentration of CF₄ for a strain among the tested strains was reduced by 10.27% in the separated microorganism. The microorganism exhibited decomposition activity of 0.02586 g/kg-cell/h. To identify the selected strain, a genome sequences thereof was analyzed.

[0068] A genome obtained by assembling 3 contigs by next generation sequencing (NGS) had a final size of 5.3 Mb, and as a result of gene annotation, a total of 5,490 genes were found to be present. As a result of phylogenetic tree analysis performed on each contig, it was confirmed that the microorganism belonged to *Bacillus bombysepticus*.

[0069] The separated microorganism was newly named as *Bacillus bombysepticus* SF3, deposited at the Korean Collection for Type Culture (KCTC), which is an international depository authority under the Budapest Treaty, on Feb. 24, 2017, and assigned the accession number of KCTC 13220BP.

Example 2: Preparation of Recombinant
Microorganism Including Gene Derived from Strain
of *B. bombysepticus* SF3, and Decomposition of
Fluorine-Containing Compound Using the
Recombinant Microorganism

[0070] 1. Preparation of Recombinant Microorganism

[0071] By the genomic sequence analysis of the strain of *B. bombysepticus* SF3 identified as described in Example 1, genes presumed to encode dehalogenase, such as GENE_00757 (SEQ ID NO: 4), GENE_01351 (SEQ ID NO: 5), and GENE_04275 (SEQ ID NO: 6), were selected.

[0072] *B. bombysepticus* SF3 was cultured overnight in an LB medium while being stirred at a temperature of 30°C at a speed of 230 rpm, and genomic DNA thereof was isolated using a total DNA extraction kit (Invitrogen Biotechnology). PCR was performed using the genomic DNA as a template and a set of primers having nucleotide sequences shown in Table 1, so as to amplify and obtain GENE_00757, GENE_01351, and GENE_04275 genes. The genes thus amplified

were each independently ligated with a pET28a vector (Novagen, Cat. No. 69864-3), using restriction enzymes, such as NcoI and XhoI, by using an InFusion Cloning Kit (Clontech Laboratories, Inc.), so as to prepare three types of pET-SF3 DEH vectors. FIG. 1 is a vector map of a pET-SF3 DEH vector. Here, GENE_00757, GENE_01351, and GENE_04275 had nucleotide sequences of SEQ ID NOs: 4, 5, and 6, respectively, and encoded amino acid sequences of SEQ ID NOs: 1, 2, and 3, respectively.

[0073] Next, each of the three prepared pET-SF3 DEH vectors (pET-SF3 00757 vector, pET-SF3 01351 vector, and pET-SF3 04275 vector) were introduced to *E. coli* BL21 by a heat shock method, and then, cultured in an LB plate agar containing 100 μg/mL of kanamycin. Strains showing kanamycin resistance were selected. Finally, three strains thus selected were designated as recombinant *E. coli* BL21/pET-SF3 00757, *E. coli* BL21/pET-SF3 01351, and *E. coli* BL21/pET-SF3 0427, respectively.

TABLE 1

SF3 DEH gene	Primer sequence (SEQ ID NO)
SF3 00757	Forward: SEQ ID NO: 7 Reverse: SEQ ID NO: 8
SF3 01351	Forward: SEQ ID NO: 9 Reverse: SEQ ID NO: 10
SF3 04275	Forward: SEQ ID NO: 11 Reverse: SEQ ID NO: 12

[0074] 2. Decomposition of Fluorine-Containing Compound Using *E. coli* Including Gene Introduced Thereto

[0075] In this section, the three of recombinant *E. coli* BL21/pET-SF3 DEH strains prepared in section (1) were examined to determine their effect on the removal of CF₄ in a sample.

[0076] In detail, each of *E. coli* BL21/pET-SF3 00757, *E. coli* BL21/pET-SF3 01351, and *E. coli* BL21/pET-SF3 04275 strains was cultured in an LB medium while being stirred at a temperature of 30°C at a speed of 230 rpm. At an OD₆₀₀ of about 0.5, 0.2 mM of IPTG was added thereto, followed by culturing at a temperature of 20°C under stirring at a speed of 230 rpm overnight. Each of the cells was harvested and suspended in a new LB medium to a cell density of OD₆₀₀ of 3.0. 10 ml of each cell suspension was added to a 60 ml-serum bottle, and then, the serum bottle was sealed. The LB medium used herein has the same composition as in Example 1.

[0077] Next, gas-phase CF₄ was injected through a rubber stopper of a cap of the serum bottle using a syringe to its headspace concentration of 1,000 ppm. Then, the serum bottle was incubated for three days while being stirred at a temperature of 30°C at a speed of 230 rpm. Here, the experiment was performed in triplicate. Following the culture, a headspace concentration of CF₄ in the serum bottle was analyzed under the same conditions as in Example 1.

[0078] Table 2 shows percentages of residual CF₄ in the samples when the recombinant *E. coli* BL21/pET-SF3 DEH strains were cultured under the conditions as above. As shown in Table 2, the recombinant *E. coli* strains introduced with GENE_00757, GENE_01351, and GENE_04275 genes showed about 9.87% decrease, about 14.41% decrease, and about 18.48% decrease in the headspace concentrations of CF₄, compared to a control group introduced with an empty vector.

TABLE 2

Strain of recombinant microorganism	Residual CF ₄ (%)
Control (empty vector)	100.00
GENE_00757	90.13
GENE_01351	85.59
GENE_04275	81.52

Example 3: Decomposition of Fluorine-Containing Compound by a Circulation Process

[0079] As shown in FIG. 2, 40 ml of an LB medium and gas-phase CF₄ at a concentration of 1,000 ppm were added to a glass Dimroth coil reflux condenser (a reactor length: 350 mm, an exterior diameter: 35 mm, and an interior volume: 200 mL) that was sterilized and vertically oriented, and the LB medium was circulated. The LB medium was first supplied to an inlet at an upper portion of the condenser, flowed through an inner wall of the condenser, and then, discharged to an outlet at a lower portion of the condenser. The discharged LB medium was re-supplied to the inlet along a circulation line. Although not shown in FIG. 2, to maintain a temperature of the condenser, a screwed pipe inside the condenser was connected to a constant temperature zone having a temperature of 30°C. Here, the LB medium was maintained at a circulation rate of 4 mL/min. After 48 hours, the amount of the gas-phase CF₄ in the condenser was confirmed by GC-MS. Accordingly, it was confirmed that there was no change in the amount of the gas-phase CF₄ in the condenser.

[0080] Subsequently, a control group and one of the strain of *B. bombysepticus* SF3 of Example 1 and one of the *E. coli* strains of Example 2 were each inoculated on an LB medium in the condenser using a syringe. Here, the control group included a wild-type strain of *Bacillus cereus*. In the LB medium on which the strains were inoculated, an initial concentration was 5.0 on the basis of OD₆₀₀. The LB culture had a circulation rate of about 4 mL/min, and the temperature inside the condenser was maintained at 30°C. Following the inoculation and after the elapse of 42, 90, and 140 hours, the amount of the gas-phase CF₄ in the condenser was confirmed by GC-MS. Here, the decomposition rate of the gas-phase CF₄ was calculated according to Equation 1, and the results are shown in FIGS. 3 to 5.

$$\text{Decomposition rate of CF}_4 = \left[\frac{\text{Initial amount of CF}_4 - \text{amount of CF}_4 \text{ after reaction}}{\text{initial amount of CF}_4} \right] \times 100 \quad \text{<Equation 1>}$$

[0081] FIG. 3 is a graph showing decomposition rates of CF₄ when a strain of *B. bombysepticus* SF3 was brought into contact with the fluorine-containing compound while being subjected to circulation in a glass Dimroth coil reflux condenser.

[0082] FIG. 4 is a graph showing decomposition rates of CF₄ when a strain of BL21/pET-SF3 00757 was brought into contact with a fluorine-containing compound while being subjected to circulation in a glass Dimroth coil reflux condenser.

[0083] FIG. 5 is a graph showing decomposition rates of CF₄ when a strain of *Bacillus cereus* was brought into contact with a fluorine-containing compound while being subjected to circulation in a glass Dimroth coil reflux condenser.

[0084] As shown in FIGS. 3 to 5, the strain of *B. bombysepticus* SF3 and the *E. coli* strain of BL21/pET-SF3 00757 showed significantly higher decomposition rates, compared to the decomposition rate of the control group.

[0085] FIG. 6 is a schematic diagram for decomposing CF₄ by applying a gas-phase circulation process using a microorganism.

[0086] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0087] The use of the terms “a” and “an” and “the” and “at least one” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term “at least one” followed by a list of one or more items (for example, “at least one of A and B”) is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0088] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

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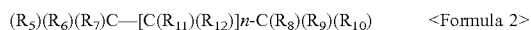
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What is claimed is:

1. A microorganism deposited with the Korean Collection for Type Culture (KCTC) under accession no. 13220BP and referred to as *Bacillus bombysepticus* SF3, which microorganism when contacted with a sample containing a fluorine-containing compound of Formula 1 or 2 reduces the concentration of the fluorine containing compound in the sample:



wherein, in Formulae 1 and 2,

n is an integer from 0 to 10,

R₁, R₂, R₃, and R₄ are each independently fluorine (F), chlorine (Cl), bromine (Br), iodine (I), or hydrogen (H), wherein at least one selected from R₁, R₂, R₃, and R₄ is F, and

R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are each independently F, Cl, Br, I, or H, wherein at least one selected from R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ is F, and

wherein, when n is equal or larger than 2, each R₁₁ is identical to or different from each other, and each R₁₂ is identical or different from each other.

2. A recombinant microorganism comprising a genetic modification that increases the level of a polypeptide or a combination thereof, the polypeptide having a sequence identity of about 90% or more with respect to an amino acid sequence of SEQ ID NO: 1, 2, or 3.

3. The recombinant microorganism of claim 2, wherein the genetic modification is an increase in copy number of a gene encoding the polypeptide.

4. The recombinant microorganism of claim 2, wherein the recombinant microorganism comprises a exogenous gene encoding the polypeptide.

5. The recombinant microorganism of claim 3, wherein the gene has a sequence identity of about 90% or more with respect to a nucleotide sequence of SEQ ID NO: 4, 5, or 6.

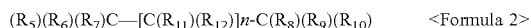
6. The recombinant microorganism of claim 2, wherein the recombinant microorganism belongs to the genus *Escherichia*, *Bacillus*, *Pseudomonas*, *Xanthobacter*, or *Saccharomyces*.

7. A composition comprising

(a) *Bacillus bombysepticus* SF3 (KCTC 13220BP) or a recombinant microorganism comprising a genetic modification that increases a level of a polypeptide that has a sequence identity of about 90% or more with respect to an amino acid sequence of SEQ ID NO: 1, 2, or 3; or a combination thereof;

and

(b) a fluorine-containing compound of Formula 1 or Formula 2:



wherein, in Formulae 1 and 2,

n is an integer from 0 to 10;

R₁, R₂, R₃, and R₄ are each independently fluorine (F), chlorine (Cl), bromine (Br), iodine (I), or hydrogen (H), wherein at least one of R₁, R₂, R₃, or R₄ is F; and

R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are each independently F, Cl, Br, I, or H, wherein at least one of R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ is F;

and wherein, when n is equal or larger than 2, each R₁₁ is identical to or different from each other, and each R₁₂ is identical to or different from each other.

8. The composition of claim 7, wherein the genetic modification is an increase in copy number of a gene encoding the polypeptide.

9. The composition of claim 7, wherein the *Bacillus bombysepticus* SF3 (KCTC 13220BP) or recombinant microorganism comprising a genetic modification that increases a level of a polypeptide that has a sequence identity of about 90% or more with respect to an amino acid sequence of SEQ ID NO: 1, 2, or 3, or both, when in contact with a sample containing a fluorine-containing compound of Formula 1 or Formula 2 has the ability to reduce the concentration of the fluorine compound in the sample, optionally, by cleaving a C—F bond of the fluorine-containing compound, converting the fluorine-containing compound into a different substance, or accumulating the fluorine-containing compound in the microorganism.

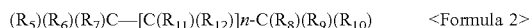
10. The composition of claim 7, wherein the fluorine-containing compound of Formula 1 or Formula 2 is in a liquid or gaseous state.

11. The composition of claim 7, wherein the recombinant microorganism belongs to the genus *Escherichia*, *Bacillus*, *Pseudomonas*, *Xanthobacter*, or *Saccharomyces*.

12. A method of reducing a concentration of a fluorine-containing compound in a sample, the method comprising:

contacting a sample comprising a fluorine-containing compound with *Bacillus bombysepticus* SF3 (KCTC 13220BP) or a recombinant microorganism comprising a genetic modification that increases a level of a polypeptide that has a sequence identity of about 90% or more with respect to the amino acid sequence of SEQ ID NO: 1, 2, or 3, or a combination thereof, so as to reduce the concentration of the fluorine-containing compound in the sample,

wherein the fluorine-containing compound is represented by Formula 1 or Formula 2:



wherein, in Formulae 1 and 2,

n is an integer from 0 to 10;

R₁, R₂, R₃, and R₄ are each independently fluorine (F), chlorine (Cl), bromine (Br), iodine (I), or hydrogen (H), wherein at least one of R₁, R₂, R₃, or R₄ is F; and

R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are each independently F, Cl, Br, I, or H, wherein at least one of R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ is F;

and wherein, when n is equal or larger than 2, each R₁₁ is identical to or different from each other, and each R₁₂ is identical to or different from each other.

13. The method of claim 12, wherein the genetic modification is an increase in copy number of a gene encoding the polypeptide.

14. The method of claim 12, wherein the contacting is performed in an air-tight sealed container.

15. The method of claim 12, wherein the contacting comprises culturing or incubating *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism while in contact with the sample.

16. The method of claim 12, wherein the contacting comprises culturing *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism under conditions in which *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism proliferates in a closed container.

17. The method of claim 12, wherein the contacting comprises, in an exhaust gas decomposition device comprising one or more reactors each of which comprises at least one first inlet and a first outlet:

injecting the sample into the exhaust gas decomposition device; and

injecting *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism through the at least one first inlet so that *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism contacts the sample and the resulting mixture is discharged through the first outlet.

18. The method of claim 17, wherein the exhaust gas decomposition device comprises a second inlet and a second outlet, the sample is injected through the second inlet and discharged through the second outlet, and a direction in which *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism moves is opposite to a direction in which the sample moves.

19. The method of claim 17, wherein a fluid thin film comprising *B. bombysepticus* SF3 (KCTC 13220BP) or the recombinant microorganism is formed on an inner wall of the one or more reactors, or on a packing material when the one or more reactors comprises a packing material.

20. A vector comprising a promoter operably linked to a nucleic acid sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 1, 2, or 3 or an amino acid sequence with at least 90% sequence identity thereto.

21. The vector of claim 20 comprising a nucleic acid sequence of SEQ ID NO: 4, 5, or 6.

22. A method of preparing a recombinant microorganism of claim 2, the method comprising introducing into a microorganism an exogenous, optionally heterologous, polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 1, 2, or 3 or an amino acid sequence with at least 90% sequence identity thereto.

23. The method of claim 22, wherein the polynucleotide comprises a nucleic acid sequence of SEQ ID NO: 4, 5, or 6.

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