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(54) **Title**: USE OF ENZYMES HAVING SILICASE ACTIVITY

(57) **Abstract**: The present invention relates to the use of polypeptides having silicase activity for the modification or synthesis of silica, silicones and other silicium (IV) compounds. The present invention also relates to the use of polypeptides having silicase activity for the modification of glass, sand, asbestos, computer chips, glass wool, fiber glass, optical fibers and silicones, for the removal of sand from oil-sands, for the removal of asbestos, and for sandblasting.

USE OF ENZYMES HAVING SILICASE ACTIVITY

Reference to a Sequence Listing

This application contains a Sequence Listing which is hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to the use of polypeptides having silicase activity for the modification or synthesis of silica, silicones and other silicium (IV) compounds and the technical use thereof.

BACKGROUND OF THE INVENTION

After oxygen, silicon is the most abundant element in the earth's crust, and it is essential for growth and biological function in a variety of plant, animal, and microbial systems (Schröder, H.C.; Krasko, A.; Le Pennec, G.; Adell, T.; Wiens, M.; Hassanein, H.; Müller, I.M.; Müller, W.E.G., Progress in Molecular and Subcellular Biology, 2003, 33, 249-268). Silicon is found in the form of free silicates ($SiO4^{x-}$, the salts of silicic acid) and bound silica (a hydrated polymer of SiO_2). Silica occurs commonly in nature as sandstone, silica sand or quartzite, wherein it is a hydrated polymer that exist in three different crystalline forms: quartz, tridymite and cristobalite. Of these, only quartz is common. Liquid silica does not readily crystallize but instead solidifies to a glass (Douglas, B.E.; Ho, S.-M. Crystal structures of silica and metal silicates. In Structure and Chemistry of Crystalline Solids, Springer: New York, 2006, 233). Silica is the starting material for the manufacture of ceramics and silicate glasses. In addition it is used as filler in a large variety of applications such as paints, plastics, rubber, adhesives, putty and sealants. In addition, a range of precious stones such as amethyst, agate, jasper, and opal are a build up of silica.

The silicates are by far the largest and the most complicated class of minerals. Approximately 30% of all minerals are silicates and some geologists estimate that 90% of the Earth's crust is made up of silicates. Examples of silicate minerals are feldspar, asbestos, clay, hornblende, and zeolites. On top of this the neosilicates (also known as orthosilicates) present a range of precious stones such as olivine, topaz, and zircon (Douglas, B.E.; Ho, S.-M. Crystal structures of silica and metal silicates. In Structure and Chemistry of Crystalline Solids, Springer: New York, 2006, 233).

Biosilicification occurs globally on a vast scale under mild conditions (e.g. neutral pH and low temperature). In fact, minute planktonic algae (diatoms) control the marine silica cycle and these single-cell plants process gigatons of particulate silica every year (Brandstadt, K.F. Curr. Opin. Biotechnol. 2005, 16, 393 and references herein). In addition to diatoms, also sponges, mollusks and higher plants can carry out biosilicification (Shimizu, K.; Cha, J.N.; Stucky, G.D.;

Morse, D.E., Proc.Natl.Acad.Sci.USA, 1998, 95, 6234 and references herein).

The synthesis of silica (biosilicification) is catalyzed by the so called silicateins (Shimizu et al. Proc.Natl.Acad.Sci.USA, 1998, 95, 6234., Zhou, Y.; Shimizu, K.; Cha, J.N.; Stucky, G.D.; Morse, D.E., Angew.Chem.Int.Ed., 1999, 38, 780. Alber, B.; Ferry, J., Proc.Natl.Acad.Sci.USA, 1994, 91, 6909) and as is usually the case in nature, enzymes with the reverse activity (silicase activity i.e. hydrolysis of silica to silicic acid) has been reported from marine sponges (e.g. Suberites domuncula), were the released silicic acid is used by other organisms for making silica skeletons (Cha, J. N.; Shimizu, K.; Zhou, Y.; Christiansen, S.C.; Chmelka, B.F.; Stucky, G.D.; Morse, D.E., Proc.Natl.Acad.Sci.USA, 1999, 96, 361).

Silicase activity has also been reported to be present as an additional activity of an alpha-carbonic anhydrase (Cha et al Proc.Natl.Acad.Sci.USA, 1999, 96, 361), Schroder et al., Progress in Molecular and Subcellular Biology, 2003, 33,249-268, and Muller et all, US Patent Publication No. 2007/0218044.

SUMMARY OF THE INVENTION

The present invention relates to the use of polypeptides having silicase activity for the modification or synthesis of silica, silicones and other silicium (IV) compounds. The present invention also relates to the use of polypeptides having silicase activity for the modification of glass, sand, asbestos, computer chips, glass wool, fiber glass, optical fibers (e.g., fictionalization of the fibers), silicones, for the separation of sand from oil-sands (e.g., by increased dissolution of the sand), for the removal of asbestos, and for sandblasting.

One aspect of the present invention relates to a method for the modification of silica, silicone and a silicium (IV) compounds, comprising treating silica, silicone or a silicium compound with a gamma-carbonic anhydrase having silicase activity.

Yet another aspect of the present invention relates to a method for the synthesis of silica, silicone and a silicium (IV) compounds, comprising treating a precursor of silica, silicone or a silicium compound with a gamma-carbonic anhydrase having silicase activity, wherein the treatment results in the synthesis of silica, silicone and a silicium (IV) compounds.

The present invention also relates to a method for the modification of silica, silicone and a silicium (IV) compounds, comprising treating silica, silicone or a silicium compound with a silicase obtained from *Methanosarcina thermophila* or a silicase activity which has a high degree of amino acid sequence identity to the *Methanosarcina thermophila* silicase (SEQ ID NO: 1).

The present invention also provides a method for the synthesis of silica, silicone and a silicium (IV) compounds, comprising treating a precursor of silica, silicone or a silicium compound with a silicase obtained from *Methanosarcina thermophila* or a silicase activity which has a degree of amino acid sequence identity to the *Methanosarcina thermophila* silicase (SEQ

ID NO: 1), wherein the treatment results in the synthesis of silica, silicone and a silicium (IV) compounds.

Another aspect of the present invention relates to a method for the modification of silica, silicone and a silicium (IV) compounds, comprising treating silica, silicone or a silicium compound with a silicase obtained from *Bacillus plakortidis*, *Bacillus clausii* or *Bacillus haludurons* or an enzyme having silicase activity which has a high degree of amino acid sequence identity to the *Bacillus plakortidis* silicase (SEQ ID NO:11 or 12), *Bacillus clausii* silicase (SEQ ID NO:9 or 10) or *Bacillus haludurons* (SEQ ID NO:8).

Yet another aspect of the present invention provides a method for the synthesis of silica, silicone and a silicium (IV) compounds, comprising treating a precursor of silica, silicone or a silicium compound with a silicase obtained from *Bacillus plakortidis*, *Bacillus clausii* or *Bacillus haludurons* or an enzyme having silicase activity which has a high degree of amino acid sequence identity to the *Bacillus plakortidis* silicase (SEQ ID NO:11 or 12), *Bacillus clausii* silicase (SEQ ID NO:9 or 10) or *Bacillus haludurons* (SEQ ID NO:8), wherein the treatment results in the synthesis of silica, silicone and a silicium (IV) compounds.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, a "silicase" or a polypeptide "having silicase activity" is an enzyme that catalyzes the inter conversion between silica and silicic acid. Silicases can hydrolyze amorphous and crystalline silicon dioxide to form free silicic acid, and due to the reversibility of the reaction, silicases can also synthesize silicone dioxide (as condensation products of silicic acid, silicates), silicones and other silicium (IV) compounds. Silicase activity may be determined according to the procedure described in Example III.

In some embodiments, polypeptides having silicase activity include polypeptides having "carbonic anhydrase" activity as well. Carbonic anhydrases (also termed "carbonate dehydratases") catalyze the inter-conversion between carbon dioxide and bicarbonate. Carbonic anhydrases are generally classified under the enzyme classification (EC 4.2.1.1). Carbonic anhydrase activity may be determined according to the procedures described in Example II

Carbonic anhydrases are widely distributed in nature in all domains of life (Smith, K. and Ferry, J. J.Bacteriol., 1999, 181, 6247; Smith, K. and Ferry, J. FEMS Microbiol.Rev., 2000, 24, 335). These enzymes have three distinct classes: the alpha-class, the beta-class and the gamma-class (Hewett-Emmett, D. and Tashian, R. Mol.Phylogenet.Evol., 1996, 5, 50). A fourth class (the delta class) has been proposed recently (So et al., J. Bacteriol., 2004, 186, 623). These classes evolved from independent origins (Bacteria, Archaea, Eukarya) with distinct protein sequence compositions, structures and functionalities. Alpha-carbonic anhydrases are abundant in all mammalian tissues where they facilitate the removal of CO_2. In prokaryotes,

genes encoding all three carbonic anhydrase classes have been identified, with the beta- and gamma-class predominating.

The inventors have discovered the presence of silicase activity in the gamma-carbonic anhydrase enzyme family, and this enzyme family may be used for the modification or synthesis of silica, silicones and other silicium (IV) compounds Examples of gamma-carbonic anhydrases having silicase activity include the gamma-carbonic anhydrase obtained from *Methanosarcina thermophila* strain TM-1 (Alber and Ferry, 1994, Proc. Natl. Acad. Sci. USA 91: 6909-6913; Alber and Ferry, 1996, J. Bacteriol. 178: 3270-3274). The amino acid sequence of the gamma-carbonic anhydrase from *Methanosarcina thermophila* is shown as SEQ ID NO:1, and is also described in WO 2008/095057. The X-ray structure of gamma-carbonic anhydrase from *Methanosarcina thermophila* has also been reported (Strop et al., J.Biol.Chem, 2001, 276, 10299).

As used herein, the term "obtained" means that the enzyme may have been isolated from an organism which naturally produces the enzyme as a native enzyme. The "obtained" enzymes may, however, be reproduced recombinantly in a host organism.

Gamma-carbonic anhydrases having silicase activity may be identified and obtained from other sources including microorganisms isolated from nature (e.g., soil, composts, water, etc.). Examples of other sources of known gamma-carbonic anhydrase include the carbonic anhydrases from *Pelobacter carbinolicus* (SEQ ID NO:2), *Syntrophus aciditrophicus* (SEQ ID NO:4), *Bacillus licheniformis* (SEQ ID NO:5), *Methanosarcina acetivorans* (SEQ ID NO:6), *Methanosarcina barkeri* (SEQ ID NO:5), *Methanosarcina mazei* (SEQ ID NO:7). The presence of silicase activity can be confirmed by the procedure described in Example III.

Gamma-carbonic anhydrases having silicase activity may also be identified and obtained from other sources including microorganisms isolated from nature (e.g., soil, composts, water, etc.) by using nucleic acid probes, e.g., as described in WO 2008/095057. Techniques for isolating microorganisms from natural habitats are well known in the art. The polynucleotide may then be obtained by similarly screening a genomic or cDNA library of another microorganism or by genome sequencing. Once a polynucleotide sequence encoding a polypeptide has been detected with the probe(s), the polynucleotide can be isolated or cloned by utilizing techniques which are well known to those of ordinary skill in the art. The presence of silicase activity can be confirmed by the procedure described in Example III.

Other silicases for use in the present invention include polypeptides having silicase activity which have a degree of identity to the *Methanosarcina thermophila* silicase (SEQ ID NO: 1) of at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%.

As used herein, the degree of "identity" between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, Trends in Genetics 16: 276-277), preferably version 3.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix (or corresponding parameters in another program used to determine % identity). The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows: (Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment).

Suitable silicase enzymes for use in the present invention include polypeptides that are substantially homologous to the *Methanosarcina thermophila* silicase (SEQ ID NO:1). "Substantially homologous polypeptides" may have one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions and other substitutions that do not significantly affect the three-dimensional folding or activity of the protein or polypeptide; small deletions, typically of one to about 30 amino acids; amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification (an affinity tag), such as a poly-histidine tract, or protein A (Nilsson et al., 1985, EMBO J. 4: 1075; Nilsson et al., 1991, Methods Enzymol. 198: 3. See, also, in general, Ford et al., 1991, Protein Expression and Purification 2: 95-107.

Suitable silicase enzymes for use in the present invention also include polypeptides that have silicase activity and differ from *Methanosarcina thermophila* silicase (SEQ ID NO:1) by up to thirty amino acids, by up to twenty-nine amino acids, by up to twenty-eight amino acids, by up to twenty-seven amino acids, by up to twenty-six amino acids, by up to twenty-five amino acids, by up to twenty-four amino acids, by up to twenty-three amino acids, by up to twenty-two amino acids, by up to twenty-one amino acids, by up to twenty-amino acids, by up to nineteen amino acids, by up to eighteen amino acids, by up to seventeen amino acids, by up to sixteen amino acids, by up to fifteen amino acids, by up to fourteen amino acids, by up to thirteen amino acids, by up to twelve amino acids, by up to eleven amino acids, by up to ten amino acids, by up to nine amino acids, by up to eight amino acid, by up to seven amino acids, by up to six amino acids, by up to five amino acids, by up to four amino acids, by up to three amino acids, by up to two amino acids, or by one amino acid.

Examples of conservative substitutions are within the group of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine,

alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, In, The Proteins, Academic Press, New York. The most commonly occurring exchanges are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly. A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, and unnatural amino acids may be substituted for amino acid residues, such as, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, and 3,3-dimethylproline.

Essential amino acids in the *Methanosarcina thermophila* silicase (SEQ ID NO:1) silicase can also be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, 1989, Science 244: 1081-1085). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (i.e., silicase activity) to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., 1996, J. Biol. Chem. 271: 4699-4708. The active site of the enzyme or other biological interaction can also be determined by physical analysis of the structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., 1992, Science 255: 306-312; Smith et al., 1992, J. Mol. Biol. 224: 899-904; Wlodaver et al., 1992, FEBS Lett. 309: 59-64. The identities of essential amino acids can also be inferred from analysis of identities with polypeptides that are related to a polypeptide according to the invention.

Single or multiple amino acid substitutions, deletions, and/or insertions can be made and tested using known methods of mutagenesis, recombination, and/or shuffling, followed by a relevant screening procedure, such as those disclosed by Reidhaar-Olson and Sauer, 1988, Science 241: 53-57; Bowie and Sauer, 1989, Proc. Natl. Acad. Sci. USA 86: 2152-2156; WO 95/17413; or WO 95/22625. Other methods that can be used include error-prone PCR, phage display (e.g., Lowman et al., 1991, Biochem. 30: 10832-10837; U.S. Patent No. 5,223,409; WO 92/06204), and region-directed mutagenesis (Derbyshire et al., 1986, Gene 46: 145; Ner et al., 1988, DNA 7: 127).

Mutagenesis/shuffling methods can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides expressed by host cells (Ness et al., 1999, Nature Biotechnology 17: 893-896). Mutagenized DNA molecules that encode active polypeptides can be recovered from the host cells and rapidly sequenced using standard methods in the art. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Silicases for use in the present invention also include fragments of the *Methanosarcina thermophila* silicase (SEQ ID NO:1) having silicase activity. A fragment of the *Methanosarcina thermophila* silicase (SEQ ID NO:1) is a polypeptide having one or more amino acids deleted from the amino and/or carboxyl terminus of this amino acid sequence. For example, the fragment may comprise SEQ ID NO:1 having a truncation at the C terminus of up to 20 amino acid residues, more preferably up to 10 amino acid residues, and most preferably up to 5 amino acid residues.

In addition to the above silicase enzymes, the present invention is further directed to the use of certain alpha-carbonic anhydrases which have also been determined to have silicase activity, in particular, the silicases from *Bacillus plakortidis* (formerly *Bacillus gibsonii*) shown as SEQ ID NO:11 or 12 which possess both silicase activity and carbonic anhydrase activity, and the silicase from *Bacillus clausii* (SEQ ID NO:10) which also posses both silicase activity and carbonic anhydrase activity. Suitable silicases also includes polypeptides having silicase activity and having a degree of identity to the *Bacillus plakortidis* silicase (SEQ ID NO: 10 or 11) or *Bacillus clausii* silicase (SEQ ID NO:9 or 10) of preferably at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%. The cloning and expression of the *B. clausii* carbonic anhydrase is described in WO 2008/09057. The preparation of the carbonic anhydrase from *B. plakortidis* is described in WO 2007/019859.

The present invention is also directed to the use the silicase from *Bacillus haludurons* shown as SEQ ID NO:8. Suitable silicases also includes polypeptides having silicase activity and having a degree of identity to the *Bacillus haludurons* (SEQ ID NO:8) of preferably at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%. The cloning and expression of the *B. haludurons* carbonic anhydrase is described in WO 2008/09057.

Suitable silicases for use in the present invention also include polypeptides having silicase activity and that differ from the silicase obtained from *Bacillus plakortidis* silicase (SEQ ID NO:11 or 12), *Bacillus clausii* silicase (SEQ ID NO:9 or 10) or *Bacillus haludurons* (SEQ ID NO:8) by up to thirty amino acids, by up to twenty-nine amino acids, by up to twenty-eight amino acids, by up to twenty-seven amino acids, by up to twenty-six amino acids, by up to twenty-five amino acids, by up to twenty-four amino acids, by up to twenty-three amino acids, by up to twenty-two amino acids, by up to twenty-one amino acids, by up to twenty-amino acids, by up to nineteen amino acids, by up to eighteen amino acids, by up to seventeen amino acids, by up to sixteen amino acids, by up to fifteen amino acids, by up to fourteen amino acids, by up to

thirteen amino acids, by up to twelve amino acids, by up to eleven amino acids, by up to ten amino acids, by up to nine amino acids, by up to eight amino acid, by up to seven amino acids, by up to six amino acids, by up to five amino acids, by up to four amino acids, by up to three amino acids, by up to two amino acids, or by one amino acid.

Silicases for use in the present invention also include fragments of the *Bacillus plakortidis* silicase (SEQ ID NO:11 or 12), *Bacillus clausii* silicase (SEQ ID NO:9 or 10) or *Bacillus haludurons* silicase (SEQ ID NO:8) having silicase activity. A fragment of the *Bacillus plakortidis* silicase (SEQ ID NO:11 or 12), *Bacillus clausii* silicase (SEQ ID NO:9 or 10) *Bacillus haludurons* silicase (SEQ ID NO:8) is a polypeptide having one or more amino acids deleted from the amino and/or carboxyl terminus of this amino acid sequence. For example, a fragment can include SEQ ID NO:8, 9, 10 or 11 truncated at the N-terminus by up to 20 amino acids, up to 10 amino acids, up to 5 amino acids.

The silicases disclosed herein for use in the present invention may be an isolated polypeptide. The term “isolated polypeptide” as used herein refers to a polypeptide that is isolated from a source. In a preferred aspect, the polypeptide is at least 1% pure, preferably at least 5% pure, more preferably at least 10% pure, more preferably at least 20% pure, more preferably at least 40% pure, more preferably at least 60% pure, even more preferably at least 80% pure, and most preferably at least 90% pure, as determined by SDS-PAGE.

The silicases disclosed herein for use in the present invention may be substantially pure. The term “substantially pure polypeptide” denotes herein a polypeptide preparation that contains at most 10%, preferably at most 8%, more preferably at most 6%, more preferably at most 5%, more preferably at most 4%, more preferably at most 3%, even more preferably at most 2%, most preferably at most 1%, and even most preferably at most 0.5% by weight of other polypeptide material with which it is natively or recombinantly associated. It is, therefore, preferred that the substantially pure polypeptide is at least 92% pure, preferably at least 94% pure, more preferably at least 95% pure, more preferably at least 96% pure, more preferably at least 96% pure, more preferably at least 97% pure, more preferably at least 98% pure, even more preferably at least 99%, most preferably at least 99.5% pure, and even most preferably 100% pure by weight of the total polypeptide material present in the preparation. The polypeptides of the present invention are preferably in a substantially pure form, i.e., that the polypeptide preparation is essentially free of other polypeptide material with which it is natively or recombinantly associated. This can be accomplished, for example, by preparing the polypeptide by well-known recombinant methods or by classical purification methods.

In accordance with the present invention, the silicases disclosed herein are used in a method for the modification of silica, silicones or silicium (IV) compounds as well as of mixed polymers of these compounds using the silicase enzymes described herein. As used herein, “modification” includes the hydrolysis or degradation of silica, silicones and other silicium (IV)

compounds.

In another embodiment of this aspect of the invention, the present invention provides a method for synthesizing silica, silicones or silicium (IV) compounds as well as of mixed polymers of these compounds using the silicase enzymes described herein. In an embodiment, the method comprises a method for the synthesis of silica, silicone and a silicium (IV) compounds, comprising treating a precursor of silica, silicone or a silicium compound with a silicase enzyme described herein, wherein the treatment results in the synthesis of silica, silicone and a silicium (IV) compounds.

The enzymes having silicase activity may be used in the modification or synthesis of a compound selected from the group consisting of such as silicic acids, monoalkoxy silantrioles, dialkoxy silandioles, trialkoxy silanoles, tetraalkoxy silanes, alkyl- or aryl-silantrioles, alkyl- or aryl-monoalkoxy silandioles, alkyl- or aryl-dialkoxy silanoles, alkyl- or aryl-trialkoxy silanes or other metal(IV)-compounds.

Technical uses of the polypeptides having silicase activity include in the modification of glass, sand, asbestos, computer chips, glass wool, fiber glass, optical fibers, and silicones. For example, modification of silica can be used for changing the surface of glass, e.g., to provide dirt repellent window glass, to adhere solar cells to window glass, to adhere sun shading materials to window glass, etc.

The polypeptides may also be used to modify the properties of fillers, such as, Sipernat and Aerosil, where other chemical groups could be attached to the polymeric silica. This functionality could also be used for the modification of silicones, where more delicate functionalization could be perform via the enzymatic reaction compared to the standard chemical reaction.

The polypeptides may also be used to separate sand from oil-sands, to get rid of waste asbestos, or to provide sandblasting under extremely mild conditions.

The silicases are used in amount effective to modify or synthesize silica, silicones or silicium (IV) compounds. The amount effective will vary depending on the technical application, and such amount can be determined by one of ordinary skill in the art. Appropriate temperature, pH and other reaction conditions can also be determined by one of ordinary skill in the art.

Silicases for use in the methods of the present invention may be formulated in any suitable form for the intended technical applications, such as, as a liquid, e.g., aqueous form, a as granulates, non-dusting granulates, or as a dry powder or as a protected enzymes.

EXAMPLES

Example I

The carbonic anhydrase gene from *Methanosarcina thermophila* (UNIPROT: P40881) was

synthetically produced and codon optimized for *Bacillus subtilis*. The gene sequence coding for the native signal peptide was exchanged to the alpha-amylase from *B. licheniformis* (AmyL) by SOE fusion as described in WO 99/43835 (hereby incorporated by reference) in frame to the DNA encoding the carbonic anhydrase. The nucleotide fragments obtained from containing the carbonic anhydrase coding sequence were integrated by homologous recombination into the *Bacillus subtilis* host cell genome. The gene construct was expressed under the control of a triple promoter system (as described in WO 99/43835). The gene coding for chloramphenicol acetyltransferase was used as maker, as described in (Diderichsen et al., 1993, Plasmid 30: 312-315).

Chloramphenicol resistant transformants were analyzed by DNA sequencing to verify the correct DNA sequence of the construct. One expression clone for each recombinant sequence was selected.

The individual carbonic anhydrase expression clones were fermented on a rotary shaking table in 1 L baffled Erlenmeyer flasks each containing 400 ml soy based media supplemented with 34 mg/l chloramphenicol. The clones were fermented for 4 days at 37°C.

The recombinant carbonic anhydrase were purified to homogeneity: The culture broth was centrifuged (26.000 x g, 20 min) and the supernatant was filtered through a Whatman 0.45 µm filter. The 0.45 µm filtrate was approx. pH 7 and conductivity was approx. 20 mS/cm. The 0.45 µm filtrate was transferred to 10 mM HEPES/NaOH, pH 7.0 by G25 sephadex chromatography and then applied to a Q-sepharose FF column. Bound protein was eluted with a linear NaCl gradient. Fractions were collected during elution and these fractions were tested for carbonic anhydrase activity.

Example II

Detection of carbonic anhydrase activity

The carbonic anhydrase activity in the culture broth and of the purified protein was determined according to (Wilbur, 1948, J Biol Chem 176: 147-154). Alternatively, the carbonic anhydrase activity was measured as esterase activity with para-nitrophenolacetate as substrate according to (Chirica et al., 2001, Biochim Biophys Acta 2001 Jan 12 ;1544 (1-2):55 -63 1544: 55-63). Details can be found in the patent application WO2008/095057 which is hereby incorporated as reference.

Example III

Silica Hydrolysis

Buffer

A buffer cocktail containing 50 mM glycine, 50 mM citric acid, 50 mM sodium phosphate,

50 mM dithiothreitol, 100 mM NaCl, and 0.5 mM $ZnSO_4$ was applied, with the pH values adjusted to 2.5, 5.0, 7.5, and 10.0 with HCl or NaOH. Buffers were made with silicate free water (Merck 1.16754.9010) in plastic containers.

Substrates

The following silica substrates were used; as a representative for crystalline silica sand (white quartz, Sigma-Aldrich 274739) was chosen, and for amorphous silica, Sipernat® 22S and Aerosil® 200 (both Degussa, now Evonik) were chosen. Sipernat and Aerosil are produced by two different methods; precipitation and pyrolysis respectively, which could give rise to differing surface properties and hence sensitivity towards enzymatic action.

Table 1. Properties of the silica forms

Silica	**Solid form**	**Surface area (m^2/g)**	**Average particle size (µm)**
Sand	Crystals	–	210 – 297
Sipernat® 22S	Amorphous	190.0	7
Aerosil® 200	Amorphous	200 ± 25	0.012

Hydrolytic activity

In 1.5 mL Eppendorf tubes 5 mg substrate and enzyme solution corresponding to 100 µg enzyme was suspended in 1.0 mL buffer. Blind determinations were run with 5 mg substrate in 1.0 mL buffer. The mixtures were incubated with shaking at room temperature (or 50°C) overnight. After 20-23 hours the suspensions were centrifuged (13.400 rpm, 15 min, 4°C), and 700 µL of the supernatant was filtered. For this, Durapore Millex-GV 22 µm 13 mm diameter filters were used.

300 µL of the filtrate was added to 4.7 mL buffer and to determine the amount of free silicic acid, the Merck Silicate Assay (1.14794) was conducted. This colorimetric assay is based on the reaction between silicate and molybdate ions to form a yellow heteropoly acid. This acid is then reduced to silicomolybdenum blue, which can be detected spectrophotometrically at 810 nm.

The absolute amounts of silicic acid were calculated after construction of a calibration curve using a silicium standard (Merck 170236). Linearity was observed from 0 to 2.5 µg silicic acid/mL. Everything was conducted in plastic containers to avoid silicate dissolution from glass.

Example IV

Hydrolysis of Aerosil® 200 by *Methanosarcina thermophila* carbonic anhydrase

Applying the experimental conditions described in Example III with Aerosil as substrate

and *M. thermophila* carbonic anhydrase as enzyme gives the results shown in Table 2.

Table 2. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.5**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.24 ± 0.03	0.26 ± 0.05	0.47 ± 0.10	2.71 ± 0.77
***Methanosarcina thermophila* carbonic anhydrase**	0.62 ± 0.05	0.85 ± 0.10	0.99 ± 0.08	5.63 ± 3.19
Net silicate formation	0.38	0.59	0.52	2.92

Example V

Hydrolysis of Aerosil® 200 by *B. clausii* carbonic anhydrase

Applying the experimental conditions described in Example IV with Aerosil as substrate and *B. clausii* carbonic anhydrase as enzyme gives the results shown in Table 3.

Table 3. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.5**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.24 ± 0.03	0.26 ± 0.05	0.47 ± 0.10	2.71 ± 0.77
***Bacillus clausii* carbonic anhydrase**	0.30 ± 0.02	0.78 ± 0.06	0.89 ± 0.07	4.36 ± 1.00
Net silicate formation	0.06	0.52	0.42	1.65

Example VI

Hydrolysis of Aerosil® 200 by *B. plakortidis* carbonic anhydrase

Applying the experimental conditions described in Example IV with Aerosil as substrate and *B. plakortidis* carbonic anhydrase as enzyme gives the results shown in Table 4.

Table 4. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.5**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.24 ± 0.03	0.26 ± 0.05	0.47 ± 0.10	2.71 ± 0.77
***Bacillus plakortidis* carbonic anhydrase**	0.18 ± 0.01	0.33 ± 0.04	0.96 ± 0.05	4.59 ± 1.74

Net silicate formation	0.06	0.07	0.50	1.88

Example VII

Hydrolysis of Sipernat® 22S by *Methanosarcina thermophila* carbonic anhydrase

Applying the experimental conditions described in Example IV with Sipernat® 22S as substrate and *M. thermophila* carbonic anhydrase as enzyme gives the results shown in Table 5.

Table 5. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.5**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.45 ± 0.13	1.25 ± 0.62	0.79 ± 0.51	3.68 ± 1.60
***Methanosarcina thermophila* carbonic anhydrase**	0.43 ± 0.07	0.86 ± 0.09	2.71 ± 0.52	8.43 ± 0.83
Net silicate formation	-0.02	-0.39	1.92	4.75

Example VIII

Hydrolysis of Sipernat® 22S by *B. clausii* carbonic anhydrase

Applying the experimental conditions described in Example IV with Sipernat® 22S as substrate and *B. clausii* carbonic anhydrase as enzyme gives the results shown in Table 6.

Table 6. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.5**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.45 ± 0.13	1.25 ± 0.62	0.79 ± 0.51	3.68 ± 1.60
***Bacillus clausii* carbonic anhydrase**	0.95 ± 0.05	0.50 ± 0.30	0.65 ± 0.12	1.45 ± 0.41
Net silicate formation	0.50	-	-	-

Example IX

Hydrolysis of Sipernat® 22S by *B. plakortidis* carbonic anhydrase

Applying the experimental conditions described in Example IV with Sipernat® 22S as

substrate and *B. plakortidis* carbonic anhydrase as enzyme gives the results shown in Table 7.

Table 7. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.5**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.45 ± 0.13	1.25 ± 0.62	0.79 ± 0.51	3.68 ± 1.60
***Bacillus plakortidis* carbonic anhydrase**	0.32 ± 0.01	0.48 ± 0.05	1.47 ± 0.27	7.49 ± 3.79
Net silicate formation	-	-	0.68	3.81

Example X

Hydrolysis of sand by *Methanosarcina thermophila* carbonic anhydrase

Applying the experimental conditions described in Example IV with sand as substrate and *M. thermophila* carbonic anhydrase as enzyme gives the results shown in Table 8.

Table 8. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.5**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.10 ± 0.01	0.05 ± 0.00	0.15 ± 0.02	0.64 ± 0.11
***Methanosarcina thermophila* carbonic anhydrase**	0.16 ± 0.003	0.23 ± 0.01	0.34 ± 0.01	0.68 ± 0.08
Net silicate formation	0.06	0.18	0.19	-

Example XI

Hydrolysis of sand by *B. clausii* carbonic anhydrase

Applying the experimental conditions described in Example IV with sand as substrate and *Bacillus clausii* carbonic anhydrase as enzyme gives the results shown in Table 9.

Table 9. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.5**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.10 ± 0.01	0.05 ± 0.00	0.15 ± 0.02	0.64 ± 0.11
***Bacillus clausii* carbonic anhydrase**	0.03 ± 0.00	0.15 ± 0.01	0.27 ± 0.03	0.69 ± 0.01

Net silicate formation	-	0.10	0.12	0.05

Example XII

Hydrolysis of sand by *B. plakortidis* carbonic anhydrase

Applying the experimental conditions described in Example IV with sand as substrate and *Bacillus plakortidis* carbonic anhydrase as enzyme gives the results shown in Table 10.

Table 10. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.5**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.10 ± 0.01	0.05 ± 0.00	0.15 ± 0.02	0.64 ± 0.11
***Bacillus plakortidis* carbonic anhydrase**	0.35 ± 0.06	0.05 ± 0.00	0.15 ± 0.01	0.64 ± 0.01
Net silicate formation	0.26	0.00	0.01	0.00

Example XIII

Hydrolysis of Glass wool by *B. clausii* carbonic anhydrase

Applying the experimental conditions described in Example III with Glass wool (Supelco, Sigma Aldrich 20384 (non-treated)) as substrate and *Bacillus clausii* carbonic anhydrase as enzyme gives the results shown in Table 11.

For the experiments at pH 10, a buffer without phosphate was used: 50 mM glycine, 50 mM dithiothreitol, 100 mM NaCl, and 0.5 mM $ZnSO_4$. pH was adjusted with HCl.

Table 11. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.8**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.48 ± 0.02	0.55 ± 0.09	0.38 ± 0.04	4.63 ± 0.28
***Bacillus clausii* carbonic anhydrase**	0.52 ± 0.04	0.63 ± 0.03	0.63 ± 0.04	6.49 ± 0.78
Net silicate formation	0.04	0.08	0.25	1.86

Example XIV

Hydrolysis of Glass wool by *B. plakortidis* carbonic anhydrase

Applying the experimental conditions described in Example III with Glass wool as substrate and *Bacillus plakortidis* carbonic anhydrase as enzyme gives the results shown in Table 12.

Table 12. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.8**	**pH 5.0**
Control	0.48 ± 0.02	0.55 ± 0.09
***Bacillus plakortidis* carbonic anhydrase**	3.37 ± 0.74	1.05 ± 0.24
Net silicate formation	2.89	0.50

Example XV

Hydrolysis of Glass wool by *Methanosarcina thermophila* carbonic anhydrase

Applying the experimental conditions described in Example III with Glass wool as substrate and *Methanosarcina thermophila* carbonic anhydrase as enzyme gives the results shown in Table 13.

For the experiments at pH 10, a buffer without phosphate was used: 50 mM glycine, 50 mM dithiothreitol, 100 mM NaCl, and 0.5 mM $ZnSO_4$. pH was adjusted with HCl.

Table 13. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.8**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.48 ± 0.02	0.55 ± 0.09	0.38 ± 0.04	4.63 ± 0.28
***Methanosarcina thermophila* carbonic anhydrase**	0.39 ± 0.02	0.36 ± 0.01	0.71 ± 0.18	3.15 ± 0.12
Net silicate formation	-0.09	-0.19	0.33	-1.48

Example XVI

Hydrolysis of Asbestos by *B. clausii* carbonic anhydrase

Applying the experimental conditions described in Example III with Asbestos (25 mg, 20% brown asbestos, Skandinavisk Bio-Medicinsk Institut A/S, Denmark) as substrate and *Bacillus clausii* carbonic anhydrase as enzyme gives the results shown in Table 14.

For the experiments at pH 10, a buffer without phosphate was used: 50 mM glycine, 50 mM dithiothreitol, 100 mM NaCl, and 0.5 mM $ZnSO_4$. pH was adjusted with HCl.

Table 14. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.8**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.39 ± 0.03	0.75 ± 0.05	0.29 ± 0.01	0.48 ± 0.03
***Bacillus clausii* carbonic anhydrase**	0.61 ± 0.05	1.57 ± 0.54	0.48 ± 0.01	0.63 ± 0.03
Net silicate formation	0.22	0.82	0.19	0.16

Example XVII

Hydrolysis of Asbestos by *Methanosarcina thermophila* carbonic anhydrase

Applying the experimental conditions described in Example III with Asbestos (25 mg, 20% brown asbestos) as substrate and *Methanosarcina thermophila* carbonic anhydrase as enzyme gives the results shown in Table 15.

For the experiments at pH 10, a buffer without phosphate was used: 50 mM glycine, 50 mM dithiothreitol, 100 mM NaCl, and 0.5 mM $ZnSO_4$. pH was adjusted with HCl.

Table 15. Silicate formation (g/l*h*g enzyme)

Silicate formation (g/l*h*g enzyme)	**pH 2.8**	**pH 5.0**	**pH 7.5**	**pH 10**
Control	0.39 ± 0.03	0.75 ± 0.05	0.29 ± 0.01	0.48 ± 0.03
***Methanosarcina thermophila* carbonic anhydrase**	0.69 ± 0.02	35.98 ± 3.89	9.95 ± 3.16	1.10 ± 0.18
Net silicate formation	0.30	35.35	9.65	0.62

SEQUENCE LISTING

Methanosarcina thermophila (SEQ ID NO:1)

MMFNKQIFTILILSLSLALAGSGCISEGAEDNVAQEITVDEFSNIRENPVTPWNPEPSAP
VIDPTAYIDPQASVIGEVTIGANVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALETI
NEEGEPIEDNIVEVDGKEYAVYIGNNVSLAHQSQVHGPAAVGDDTFIGMQAFVFKSKVGN
NCVLEPRSAAIGVTIPDGRYIPAGMVVTSQAEADKLPEVTDDYAYSHTNEAVVYVNVHLA
EGYKETS

Bacillus licheniformis (SEQ ID NO:2)

MKLSSKLILGLTVSSLAGKFLEKLLIQDNVSPNITASFNQEADIPDIDASSYIHHFASVI
GSVVIGRNVFIGPFSSIRGDVGLKIFISHDCNIQDGVVLHGLKNYEYNSPVTEHSVFKDR
ESYSIYIGEKVSLAPQCQIYGPVRIDKNVFVGMQSLVFDAYIQEDTVIEPGAKIIGVTIP
PKRFVSAGRVISNQEDANRLPEITDSYPYHDLNSKMTSVNLELAKGYKKEERQWKL

Pelobacter carbinolicus (SEQ ID NO:3)

MIEKNVVTDFCSEASEPVIDASTYVHPLAAVIGNVILGKNIMVSPTAVVRGDEGQPLHVG
DDSNIQDGVVIHALETEMNGKPVAKNLYQVDGRSYGAYVGCRVSLAHQVQIHGPAVVLDD
TFVGMKSLVFKSFVGKGCVIEPGSIVMGVTVADGRYVPAGSVIRTQEDADALPEIGADYP
FRAMNPGVVHVNTALAKGYMVKQGN

Syntrophus aciditrophicus (SEQ ID NO:4)

MIGKNVLTDFSARASEPVIGSFTFVHPLAAVIGNVILGDNIMVSPGASIRGDEGQPLYVG
SDSNVQDGVVIHALETELDGKPVEKNLVEVDGKKYAVYVGNRVSLAHQVQVHGPAVIRDD
TFVGMKSLVFKSYVGSNCVIEPGVLLMGVTVADGRYVPAGSVVKTQEQADALPVITDDYP
MKEMNKGVLHVNKALARGYLAAGS

Methanosarcina barkeri (SEQ ID NO:5)

MRFNKQTFTILILSLSLALLGSGCISEGEGAEGNVTQGITESEFSNIRENPVTPWNPVPV
APVIDPTAFIDPQASVIGNVTIGASVMVSPMASIRSDEGMPIFVGDRSNVQDGVVLHALE
TIDEEGEPVENNIVEVGGKKYAVYIGENVSLAHQAQVHGPASVGNDTFIGMQAFVFKSKI
GNNCVLEPTSAAIGVTVPDGRYIPAGMVVTSQAEADNLSEITDDYAYKHTNEAVVYVNVH

LAEGYNKA

Methanosarcina acetivorans (SEQ ID NO:6)

MKINRIFLALLFSLALTLAGSGCVSQGEGAEDGESADTEVESEVSNIRANPVTPWNPEPT
EPVIDSTAYIHPQAAVIGDVTIGASVMVSPMASVRSDEGTPIFVGDETNIQDGVVLHALE
TVNEEGEPVESNLVEVDGEKYAVYVGERVSLAHQSQIHGPAYVGNDTFIGMQALVFKANV
GDNCVLEPKSGAIGVTIPDGRYIPAGTVVTSQAEADELPEVTDDYGYKHTNEAVVYVNVN
LAAGYNA

Methanosarcina mazei (SEQ ID NO:7)

MALLLSLAITLAGSGCVSQGEGAEEGENIEAEEVEANVEESNIRANPVTPWNPEPTEPVI
DPTAYIHPQASVIGDVTIGASVMVSPMASVRSDEGMPIFVGDECNIQDGVILHALETVNE
EGEPVEENQVEVDGKKYAVYIGERVSLAHQAQVHGPSLVGNDTFIGMQTFVFKAKIGNNC
VLEPTSAAIGVTVPDGRYIPAGTVVTSQDEADKLPEVTDDYAYKHTNEAVVYVNTNLAEG
YNA

Bacillus halodurans (SEQ ID NO:8)

MKKYLWGKTCLVVSLSVMVTACSSAPSTEPVDEPSETHEETSGGAHEVHWSYTGDTGPEH
WAELDSEYGACAQGEEQSPINLDKAEAVDTDTEIQVHYEPSAFTIKNNGHTIQAETTSDG
NTIEIDGKEYTLVQFHFHIPSEHEMEGKNLDMELHFVHKNENDELAVLGVLMKAGEENEE
LAKLWSKLPAEETEENISLDESIDLNALLPESKEGFHYNGSLTTPPCSEGVKWTVLSEPI
TVSQEQIDAFAEIFPDNHRPVQPWNDRDVYDVITE

Bacillus clausii (SEQ ID NO:9)

MKRSHLFTSITLASVVTLATAPAASAASFLSPLQALKASWSYEGETGPEFWGDLDEAFAA
CSNGKEQSPINLFYDREQTSKWNWAFSYSEAAFSVENNGHTIQANVENEDAGGLEINGEA
YQLIQFHFHTPSEHTIEETSFPMELHLVHANHAGDLAVLGVLMEMGNDHEGIEAVWEVMP
EEEGTAAYSISLDPNLFLPESVTAYQYDGSLTTPPCSEGVKWTVLNDTISISETQLDAFR
DIYPQNYRPVQELGDREIGFHYH

Bacillus clausii (SEQ ID NO:10)

ASAASFLSPLQALKASWSYEGDTGPEFWGDLDEAFAACSNGKEQSPINLFYDREQTPKWN

WAFSYSEAAFSVENNGHTIQANVENEDAGGLEINGEAYQLTQFHFHTPSEHTIEETSFPM
ELHLVHANHAGDLAVLGVLMEIGNDHEGIEAVWEVMPEEEGTAEYSISIDPSLFLPESVT
AYQYDGSLTTPPCSEGVKWTVLNDTISISATQLDAFRAIYPQNYRPVQELGDREIGFHYH

Bacillus gibsonii (SEQ ID NO:11)

MSKMKTLRKLSLYAAFTLSSSSMVTAPLFAQTDTHQPLIPYHSLHLLLHSNEKEGWSYSG
STGPQFWADLHDEYIACSQGKEQSPVALHNEDASDEGKWSLDLDYNETDFSIENNGHTIQ
ANVGDHSSNKLIVNGTDYKLAQFHFHSQSEHTLDDDYYEMELHLVHQDEEDNLAVLGVLI
EEGEKNETLANMWDVIPETEGEADETISLNPSELVPKDPLVSTCRRASSSFCSL

Bacillus gibsonii (SEQ ID NO:12)

MRKTNVLLKSTMITTLLLSSTLLVTTPIYAHTETQQLSYKTLNDLLTEDGHSGWSYSGLT
GPEYWGELNEDYKACSKGEEQSPIALQNEDIDNEKWSFDLDYEETEFSIENNGHTIQANV
EGSSSNTLLLNDTEYNLVQFHFHSPSEHTLDDEYFEMEVHLVHQDEHANLAVLGVLIEEG
EQNETLTDMWELMPGQQGEAAERITLNPSELVPSDLSTFQYDGSLTTPPCSEDVKWSVSD
STISLSPEQLEAFQDLYPNNYRPIQDLGNREVGFHY

CLAIMS

1. A method for the modification of silica, silicone and a silicium (IV) compound, comprising treating silica, silicone or a silicium compound with a gamma-carbonic anhydrase having silicase activity.

2. A method for the synthesis of silica, silicone and a silicium (IV) compound, comprising treating a precursor of silica, silicone or a silicium compound with a gamma-carbonic anhydrase having silicase activity, wherein the treatment results in the synthesis of silica, silicone and a silicium (IV) compound.

3. A method for the modification of silica, silicone and a silicium (IV) compound, comprising treating silica, silicone or a silicium compound with a silicase obtained from *Methanosarcina thermophila*, or a silicase activity which has a degree of identity to the *Methanosarcina thermophila* silicase (SEQ ID NO:1) of at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%.

4. The method of claim 3, wherein the silicase is obtained from *Methanosarcina thermophila.*

5. A method for the synthesis of silica, silicone and a silicium (IV) compound, comprising treating a precursor of silica, silicone or a silicium compound with a silicase obtained from *Methanosarcina thermophila*, or a silicase activity which has a degree of identity to the *Methanosarcina thermophila* silicase (SEQ ID NO:1) of at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, wherein the treatment results in the synthesis of silica, silicone and a silicium (IV) compound.

6. The method of claim 5, wherein the silicase is obtained from *Methanosarcina thermophila.*

7. A method for the modification of silica, silicone and a silicium (IV) compound, comprising treating silica, silicone or a silicium compound with a silicase obtained from *Bacillus plakortidis*, *Bacillus clausii* or *Bacillus haludurons*, or a silicase activity which has a degree of identity to the *Bacillus plakortidis* silicase (SEQ ID NO:11 or 12), *Bacillus clausii* silicase (SEQ ID NO:9 or 10) or *Bacillus haludurons* silicase (SEQ ID NO:8) of at least 60%, more preferably at least 65%,

more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%.

8. The method of claim 7, wherein the silicase is obtained from *Bacillus plakortidis*.

9. The method of claim 7, wherein the silicase is obtained from *Bacillus clausii*.

10. The method of claim 7, wherein the silicase is obtained from *Bacillus haludurons.*

11. A method for the synthesis of silica, silicone and a silicium (IV) compound, comprising treating a precursor of silica, silicone or a silicium compound with a silicase obtained from *Bacillus plakortidis, Bacillus clausii* or *Bacillus haludurons*, or a silicase activity which has a degree of identity to the *Bacillus plakortidis* silicase (SEQ ID NO:11 or 12), *Bacillus clausii* silicase (SEQ ID NO:9 or 10) or *Bacillus haludurons* silicase (SEQ ID NO:8) of at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, wherein the treatment results in the synthesis of silica, silicone and a silicium (IV) compound.

12. The method of claim 11, wherein the silicase is obtained from *Bacillus plakortidis*.

13. The method of claim 11, wherein the silicase is obtained from *Bacillus clausii*.

14. The method of claim 11, wherein the silicase is obtained from *Bacillus haludurons.*

15. The method of any one of claims 1-14, wherein the silicase is used for the modification of glass, sand, asbestos, computer chips, glass wool, fiber glass, optical fibers and silicones, for the removal of sand from oil-sands, for the removal of asbestos, or for sandblasting.