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(54) Title: CO_2 CAPTURE METHODS USING THERMOVIBRIO AMMONIFICANS CARBONIC ANHYDRASE

(57) Abrégé/Abstract:
Use of Thermovibrio ammonificans carbonic anhydrase (TACA) or mutants thereof for catalyzing the hydration reaction of CO_2 into bicarbonate and hydrogen ions or catalyzing the desorption reaction to produce a CO_2 gas.

ABSTRACT

Use of *Thermovibrio ammonificans* carbonic anhydrase (TACA) or mutants thereof for catalyzing the hydration reaction of CO_2 into bicarbonate and hydrogen ions or catalyzing the desorption reaction to produce a CO_2 gas.

CO_2 CAPTURE METHODS USING *THERMOVIBRIO AMMONIFICANS* CARBONIC ANHYDRASE

TECHNICAL FIELD

The technical field relates to CO_2 capture and the use of *Thermovibrio ammonificans* carbonic anhydrase (TACA) and /or mutants for catalyzing the hydration reaction of CO_2 into bicarbonate and hydrogen ions or catalyzing the desorption reaction to produce a CO_2 gas.

BACKGROUND

Increasingly dire warnings of the dangers of climate change by the world's scientific community combined with greater public awareness and concern over the issue has prompted increased momentum towards global regulation aimed at reducing man-made greenhouse gas (GHGs) emissions, most notably carbon dioxide. Ultimately, a significant cut in North American and global CO_2 emissions will require reductions from the electricity production sector, the single largest source of CO_2 worldwide. According to the International Energy Agency's (IEA) GHG Program, as of 2006 there were nearly 5,000 fossil fuel power plants worldwide generating nearly 11 billion tons of CO_2, representing nearly 40% of total global anthropogenic CO_2 emissions. Of these emissions from the power generation sector, 61% were from coal fired plants. Although the long-term agenda advocated by governments is replacement of fossil fuel generation by renewables, growing energy demand, combined to the enormous dependence on fossil generation in the near term dictates that this fossil base remain operational. Thus, to implement an effective GHG reduction system will require that the CO_2 emissions generated by this sector be mitigated, with carbon capture and storage (CCS) providing one of the best known solutions.

The CCS process removes CO_2 from a CO_2 containing gas and involves the production of a highly concentrated CO_2 gas stream which is compressed and transported to a geologic sequestration site. This site may be a depleted oil field, a saline aquifer or any suitable storage site. Sequestration in oceans and mineral carbonation are two alternate ways to sequester CO_2 that are in the research phase. Captured CO_2 can also be used for enhanced oil recovery or for carbonation of alkaline waste streams for sequestration as mineral solids.

Conventional technologies for CO_2 capture are based on the use of aqueous amines (e.g. alkanolamines) and carbonates solutions which are circulated through two main distinct units: an absorption unit coupled to a desorption (or stripping) unit. However in the context of low CO_2 partial pressures encountered in gases from combustion, these conventional technologies give rise to processes with high energy penalty and thus high operational expenditure, as it is the case with monoethanolamine (MEA), or processes with high capital expenditure, as for the case of kinetically limited absorption solutions resulting in large equipment such as with methydiethanolamine (MDEA) for example. Higher pressure CO_2 separation from process streams seen in H_2 production or gasification is typically usually easier to achieve due to the higher pressures in such processes.

Carbonic anhydrase is an enzyme that has been used for CO_2 absorption applications. Carbonic anhydrase is not just a single enzyme form, but a broad group of metalloproteins that exists in genetically unrelated families of isoforms, α, β, γ, δ and ε. Different classes, isoforms and variants of carbonic anhydrase have been used in order to catalyze the hydration reaction of CO_2 into bicarbonate and hydrogen ions and the bicarbonate dehydration reaction into CO_2 and water, as follows:

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^- \text{ (Reaction 1)}$$

Under optimum conditions, the catalyzed turnover rate of the hydration reaction can reach 1×10^6 molecules/second.

However, there are several challenges related to the use of carbonic anhydrase in CO_2 capture operations. For instance, the temperature stability in time, the chemical resistance and the activity of the carbonic anhydrase under process conditions are factors that have an impact on process design, process performance and operating costs.

There is thus a need to overcome at least some of the challenges related to the use of carbonic anhydrase for CO_2 capture.

SUMMARY

The present invention provides a polynucleotide comprising a nucleotide sequence encoding the carbonic anhydrase polypeptide of the invention.

In some aspects, there is an expression or cloning vector comprising a nucleotide sequence encoding the carbonic anhydrase polypeptide as defined therein.

In some aspects, there is a transgenic cell comprising the expression or cloning vector as defined therein.

The present invention provides various methods or techniques related to the use of the carbonic anhydrase polypeptide as defined therein for removing CO_2 from a CO_2-containing effluent.

The present invention provides various methods or techniques related to the use of TA carbonic anhydrase (TACA) for CO_2 capture and/or catalyzing the absorption of CO_2 from a gas into a liquid phase.

In some aspects, there is a use of the carbonic anhydrase polypeptide comprising the sequence as set forth in SEQ ID NO 2, 4 or 6.

In some aspects, there is a method for absorbing CO_2 from a CO_2-containing gas, comprising:

contacting the CO_2-containing gas with an aqueous absorption solution to dissolve the CO_2 into the aqueous absorption solution; and

providing the *Thermovibrio ammonificans* carbonic anhydrase (TACA) described therein to catalyze the hydration reaction of the dissolved CO_2 into bicarbonate and hydrogen ions.

In some aspects, there is a method for absorbing CO_2 from a CO_2-containing gas, comprising: contacting the CO_2-containing gas with an aqueous absorption solution to dissolve the CO_2 into the aqueous absorption solution; providing a *Thermovibrio ammonificans* carbonic anhydrase (TACA) or functional derivative thereof to catalyze the hydration reaction of the dissolved CO_2 into bicarbonate and hydrogen ions; and providing operating conditions such that the TACA displays enhanced stability and/or activity.

In some aspects, the enzyme of the invention is able to operate under temperature conditions advantageous for the process while lasting for a long period of time, in order to minimize the enzyme consumption during operation.

In some aspects, the method of the invention for absorbing CO_2 from a CO_2-containing gas, comprises the use of TACA of SEQ ID NO 2 or SEQ ID NO 4: or SEQ ID NO 6.

In some aspects, the TACA provides an enhanced CO_2 flux of at least 8.5 times a corresponding CO_2 flux with no enzyme.

In some aspects, the invention provides a method described therein, wherein the aqueous absorption solution comprises at least one absorption compound.

In some aspects, the invention provides a method described therein, wherein the absorption solution comprises at least one absorption compound and the at least absorption compound comprises a primary amine, a secondary amine, a tertiary amine, a primary alkanolamine, a secondary alkanolamine, a tertiary alkanolamine, a primary amino acid, a secondary amino acid, a tertiary amino acid, dialkylether of polyalkylene glycols, dialkylether or dimethylether of polyethylene glycol, amino acid or a derivative thereof, monoethanolamine (MEA), 2-amino-2-methyl-1-propanol (AMP), 2-(2-aminoethylamino)ethanol (AEE), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris or AHPD), N-methyldiethanolamine (MDEA), dimethylmonoethanolamine (DMMEA), diethylmonoethanolamine (DEMEA), triisopropanolamine (TIPA), triethanolamine (TEA), DEA, DIPA, MMEA, TIA, TBEE, HEP, AHPD, hindered diamine (HDA), bis-(tertiarybutylaminoethoxy)-ethane (BTEE), ethoxyethoxyethanol-tertiarybutylamine (EEETB), bis-(tertiarybutylaminoethyl)ether, 1,2-bis-(tertiarybutylaminoethoxy)ethane and/or bis-(2-isopropylaminopropyl)ether, or a combination thereof.

In some aspects, the invention provides a method described therein, wherein the at least one absorption compound comprises a primary amine, a secondary amine, a tertiary amine, a primary alkanolamine, a secondary alkanolamine, a tertiary alkanolamine, a primary amino acid, a secondary amino acid, a tertiary amino acid or a combination thereof.

In some aspects, the invention provides a method described therein, wherein the at least one absorption compound comprises dialkylether of polyalkylene glycols, dialkylether or dimethylether of polyethylene glycol, amino acid or derivative thereof or a combination thereof.

In some aspects, the invention provides a method described therein, wherein the at least one absorption compound comprises piperazine or derivatives thereof.

In some aspects, the invention provides a method described therein, wherein the piperazine or derivatives thereof are substituted by at least one of alkanol group.

In some aspects, the invention provides a method described therein, wherein the at least one absorption compound comprises monoethanolamine (MEA), 2-amino-2-methyl-1-propanol (AMP), 2-(2-aminoethylamino)ethanol (AEE), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris or AHPD), N-methyldiethanolamine (MDEA), dimethylmonoethanolamine (DMMEA), diethylmonoethanolamine (DEMEA), triisopropanolamine (TIPA), triethanolamine (TEA), DEA, DIPA, MMEA, TIA, TBEE, HEP, AHPD, hindered diamine (HDA), bis-(tertiarybutylaminoethoxy)-ethane (BTEE), ethoxyethoxyethanol-tertiarybutylamine (EEETB), bis-(tertiarybutylaminoethyl)ether, 1,2-bis-(tertiarybutylaminoethoxy)ethane and/or bis-(2-isopropylaminopropyl)ether.

In some aspects, the invention provides a method described therein, wherein the at least one absorption compound comprises an amino acid or derivative thereof.

In some aspects, the invention provides a method described therein, wherein the amino acid or derivative thereof comprises glycine, proline, arginine, histidine, lysine, aspartic acid, glutamic acid, methionine, serine, threonine, glutamine, cysteine, asparagine, valine, leucine, isoleucine, alanine, tyrosine, tryptophan, phenylalanine, taurine, N,cyclohexyl 1,3-propanediamine, N-secondary butyl glycine, N-methyl N-secondary butyl glycine,diethylglycine, dimethylglycine, sarcosine, methyl taurine, methyl-α-aminopropionicacid, N-(β-ethoxy)taurine, N-(β-aminoethyl)taurine, N-methyl alanine, 6-aminohexanoic acid, potassium or sodium salt of the amino acid or a combination thereof.

In some aspects, the invention provides a method described therein, wherein the absorption compound comprises a carbonate compound.

In some aspects, the invention provides a method described therein, wherein the absorption compound comprises sodium carbonate, potassium carbonate or MDEA.

In some aspects, the invention provides a method described therein, wherein the absorption compound comprises sodium carbonate.

In some aspects, the invention provides a method described therein, wherein the absorption compound comprises potassium carbonate.

In some aspects, the invention provides a method described therein, wherein the temperature of the absorption solution is at least 10°C.

In some aspects, the invention provides a method described therein, wherein the temperature of the absorption solution is at least 25°C.

In some aspects, the step of contacting is performed at a temperature between about 10°C and about 98°C, between about 25°C and about 80°C, between about 30°C and about 70°C, or between about 40°C and about 50°C, optionally at 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80 °C or 98°C or any other value in between. The absorption solution may include an absorption compound, which may include sodium or potassium carbonate.

In some aspects, the concentration of the TACA or functional derivative is between about 0.01 g/L and about 50 g/L, optionally between about 0.3g/L and about 10 g/L, in the absorption solution.

In some aspects, the pH of the absorption solution is between about 8 and about 11.

In some aspects, the CO_2 loading is between about 0.05 and about 1 mol CO_2/mol amine or mol CO_2/mol cation.

In some aspects, the method described therein further comprises subjecting the ion-rich solution to desorption to produce a regenerated absorption solution and a CO_2 gas stream.

In some aspects, at least a portion of the TACA or functional derivative is a component of the absorption solution and the ion-rich solution and catalyzes the desorption reaction.

In some aspects, the absorption is operated at a temperature between about 10°C and about 98°C, optionally between about 25°C and about 80°C, between about 30°C and about 70°C, or between about 40°C and about 50°C, optionally at 10°C, 20°C, 30°C, 40°C, 50°C,

60°C, 70°C, 80 °C or 98°C or any other value in between. Absorption operation can be operated under a wide range of pressure from 1 to 100 bar.

In some aspects, the desorption is operated at a temperature between about 30^0C and about 110^0C, optionally between about 35^0C and about 90^0C or between about 40^0C and about 70^0C. Desorption operation can be operated under a wide range of pressure from 0.05 bar up to 50 bars.

In some aspects, the absorption solution includes at least one absorption compound. The at least one absorption compound may include a primary amine, a secondary amine, a tertiary amine, a primary alkanolamine, a secondary alkanolamine, a tertiary alkanolamine, a primary amino acid, a secondary amino acid, a tertiary amino acid, a carbonate or a combination thereof. The at least one absorption compound may include dialkylether of polyalkylene glycols, dialkylether or dimethylether of polyethylene glycol, amino acid or derivative thereof or a combination thereof. The at least one absorption compound may include piperazine or derivative thereof, which may be substituted by at least one of alkanol group. The at least one absorption compound may include monoethanolamine (MEA), 2-amino-2-methyl-1-propanol (AMP), 2-(2-aminoethylamino)ethanol (AEE), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris), N-methyldiethanolamine (MDEA), dimethylmonoethanolamine (DMMEA), diethylmonoethanolamine (DEMEA), triisopropanolamine (TIPA), triethanolamine (TEA), DEA, DIPA, methyl monoethanolamine (MMEA), TIA, TBEE, HEP, AHPD, hindered diamine (HDA), bis-(tertiarybutylaminoethoxy)-ethane (BTEE), ethoxyethoxyethanol-tertiarybutylamine (EEETB), bis-(tertiarybutylaminoethyl)ether, 1,2-bis-(tertiarybutylaminoethoxy)ethane and/or bis-(2-isopropylaminopropyl)ether. The at least one absorption compound may include an amino acid or derivative thereof, which may include glycine, proline, arginine, histidine, lysine, aspartic acid, glutamic acid, methionine, serine, threonine, glutamine, cysteine, asparagine, valine, leucine, isoleucine, alanine, tyrosine, tryptophan, phenylalanine, taurine, N,cyclohexyl 1,3-propanediamine, N-secondary butyl glycine, N-methyl N-secondary butyl glycine,diethylglycine, dimethylglycine, sarcosine, methyl taurine, methyl-α-aminopropionicacid, N-(β-ethoxy)taurine, N-(β-aminoethyl)taurine, N-methyl alanine, 6-aminohexanoic acid, potassium or sodium salt of the amino acid, sodium carbonate, potassium carbonate or a combination thereof.

In some aspects, the method further includes subjecting the ion-rich solution to desorption to produce a regenerated absorption solution and a CO_2 gas stream. At least a portion of the TACA may be a component of the absorption solution and the ion-rich solution and catalyzes the desorption reaction.

In some aspects, there may be a method for CO_2 capture, including:

in an absorption stage:

contacting a CO_2-containing gas with an aqueous absorption solution to dissolve the CO_2 into the aqueous absorption solution;

providing *Thermovibrio ammonificans* carbonic anhydrase (TACA) of the invention or functional derivative thereof in the absorption solution to catalyze the hydration reaction of the dissolved CO_2 into bicarbonate and hydrogen ions, thereby producing an ion-rich solution comprising at least some of the TACA and a CO_2-depleted gas;and/or

in a desorption stage:

providing conditions for treating the ion-rich solution comprising at least some of the TACA of the invention, or functional derivative thereof, so as to desorb CO_2 gas from the ion-rich solution, thereby producing a regenerated absorption solution and a CO_2 gas stream.

In some aspects, the absorption stage may be operated with at least one of the following absorption operating parameters:

absorption temperature in between about 10°C and about 98°C;

concentration of an absorption compound in the absorption solution between about 0.1M and about 5M;

pH of the absorption solution in between about 8 and about 11; and/or

CO_2 loading in between about 0.05 and about 1 mol CO_2/mol amine or mol CO_2/mol cation.

In some aspects, the desorption stage is operated with the following desorption operating parameter: desorption temperature in between about 30°C and about 110°C.

In some aspects, the absorption stage and desorption stage are operated within an overall operating temperature zone wherein the TACA or functional derivative shows 100% residual activity after at least 1 week of exposure to overall operating temperature zone.

In some aspects, the absorption stage and desorption stage are operated within an overall operating temperature zone wherein the TACA or functional derivative provides enhanced temperature stability compared to a reference enzyme.

The absorption stage and desorption stage may be operated within an overall operating temperature zone wherein the TACA or functional derivative thereof displays enhanced temperature stability and/or activity and/or an overall enhancement of the use of the enzyme.

The absorption stage and desorption stage are operated within an overall operating temperature zone wherein the TACA or functional derivative thereof displays enhanced temperature stability.

In some aspects, there is a method for desorption of CO_2 from a solution comprising bicarbonate and hydrogen ions, comprising providing conditions for desorption of the CO_2 in the presence of a *Thermovibrio ammonificans* carbonic anhydrase (TACA) or functional derivative thereof, so as to catalyze the desorption of CO_2 gas from the solution, thereby producing an ion-depleted solution and a CO_2 gas stream.

In some aspects, there is a method for stripping CO_2 from a bicarbonate-containing aqueous absorption solution, comprising: contacting the bicarbonate-containing solution with a CO_2 free gas to transform the bicarbonate ion back into CO_2 in the absorption solution and desorb it so it is transferred into the gas; providing a *Thermovibrio ammonificans* carbonic anhydrase (TACA) or functional derivative thereof to catalyze the dehydration reaction of the bicarbonate and hydrogen ions into CO_2 and water; and providing operating conditions such that the TACA or functional derivative displays enhanced stability and/or activity.

In some aspects, there is a system for absorbing CO_2 from a CO_2-containing gas, comprising:

an absorption unit comprising:

a gas inlet for receiving the CO_2-containing gas;

a liquid inlet for receiving an aqueous absorption solution;

a reaction chamber for contacting the CO_2-containing gas with the aqueous absorption solution to dissolve the CO_2 into the aqueous absorption solution, wherein *Thermovibrio ammonificans* carbonic anhydrase (TACA) or functional derivative thereof is present for catalyzing the hydration reaction of the dissolved CO_2 into bicarbonate and hydrogen ions, thereby producing an ion-rich solution and a CO_2-depleted gas;

a liquid outlet for releasing the ion-rich solution; and

a gas outlet for releasing the CO_2-depleted gas.

The system of the invention may further include a regeneration stage for regenerating the ion-rich solution. The regeneration stage may include a desorption unit and/or a mineralization unit.

The system of the invention may also include a temperature regulator for regulating the temperature of the absorption unit to promote enhanced stability and/or activity of the TACA or functional derivative thereof.

In some aspects, the invention provides a method, wherein the operating conditions are provided such that the combined stability and activity of the TACA or functional derivative provide enhanced overall CO_2 capture over time per given enzyme utilization.

In some aspects, the invention provides the system, method or use described therein, wherein the operating conditions are provided such that the combined stability and activity of the TACA or functional derivative thereof provide enhanced overall CO_2 capture over time per given enzyme utilization.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig 1 shows an amino acid sequence SEQ ID NO 2 of TACA and its nucleic acid encoding sequence SEQ ID NO 1. The cleaved signal peptide is underscored and may be replaced with a methionine. DNA sequence taken from NCBI Reference Sequence: NC_014926.1

Fig 2 shows sequence similarities between TACA and the most similar proteins in GenBank, which were located by performing a protein Blast against known sequences in GenBank.

Fig 3 is a graph of residual activity of various carbonic anhydrases, including TACA, after 16 hours incubation in 1.45M $KHCO_3/K_2CO_3$ pH 10 (2.9M K^+) at various temperatures.

Fig 4 is a graph of residual activity of various carbonic anhydrases, including TACA, after various incubation times in 1.45M $KHCO_3/K_2CO_3$ pH 10 (2.9M K^+) at 60°C.

Fig 5 is a graph of residual activity of various carbonic anhydrases, including TACA, after various incubation times in 1.45M $KHCO_3/K_2CO_3$ pH 10 (2.9M K^+) at 75°C.

Fig 6 is a graph of residual activity of various carbonic anhydrases, including TACA, after various incubation times in 1.45M $KHCO_3/K_2CO_3$ pH 10 (2.9M K^+) at 85°C.

Fig 7 is a graph of residual activity of various carbonic anhydrases, including TACA, after a 1 hour incubation in 1.45M $KHCO_3/K_2CO_3$ pH 10 (2.9M K^+) at 98°C.

Fig 8 is a graph of residual activity of TACA after different thermal cycling times in 1.45M $KHCO_3/K_2CO_3$ pH 10 (2.9M K^+). Temperature profile for one cycle is given in Fig 9. One cycle lasts 8 minutes and is repeated 180 times per day. A total of 28 days was performed, representing a sum of 5040 cycles. Different enzyme concentrations were tested.

Fig 9 is related to thermal cycling described in Fig 8 and shows temperature fluctuations occuring in one cycle representative of a CO_2 capture process.

Fig 10 is a graph of residual activity of various carbonic anhydrases, including TACA, after various incubation times in 20% MDEA alpha=0.1 (mol CO_2 /mol MDEA) at 60°C

Fig 11 is a process flow diagram illustrating one embodiment of the present invention, using a CO_2 capture system.

Fig 12 is another process flow diagram illustrating one embodiment of the present invention, using a CO_2 capture system including a separation unit.

Fig 13 shows a polynucleotide sequence SEQ ID NO 3 encoding TACA without its signal peptide. The ATG codon, encoding methionine, replaced the signal peptide encoding sequence.

Fig 14 shows a polypeptide sequence SEQ ID NO 4 corresponding to TACA without its signal peptide. A methionine replaces the signal peptide.

Fig 15 shows a polynucleotide sequence SEQ ID NO 5 encoding TACA, without its signal peptide, and where the first five amino acids were replaced by the GLU-HIS-GLU sequence.

Fig 16 shows a polypeptide sequence SEQ ID NO 6 corresponding to TACA without its signal peptide and where the first five amino acids where replaced by the GLU-HIS-GLU sequence.

DETAILED DESCRIPTION

Various methods or techniques are provided herein for CO_2 capture using TACA for catalysis, leveraging the stability and activity of the TACA for operating conditions of the CO_2 capture process.

TACA is a carbonic anhydrase that catalyzes the interconversion of CO_2 and water to bicarbonate and hydrogen ions or vice versa. TACA is obtained or derived from the thermophilic bacteria *Thermovibrio ammonificans* (TA) (Giovannelli D, Ricci J, Pérez-Rodríguez I, Hügler M, O'Brien C, Keddis R, Grosche A, Goodwin L, Bruce D, Davenport KW, Detter C, Han J, Han S, Ivanova N, Land ML, Mikhailova N, Nolan M, Pitluck S, Tapia R, Woyke T, Vetriani C. "Complete genome sequence of *Thermovibrio ammonificans* HB-1(T), a thermophilic, chemolithoautotrophic bacterium isolated from a deep-sea hydrothermal vent" Standards in Genomic Science 2012 7:82-90.). Methods for isolating/obtaining an enzyme from bacteria are known, such as immunoprecipitation,

ultracentrifugation or chromatographic methods. Further details and definitions related to TACA may be found in the Definitions section below. TA grows in the temperature range of 60°C to 80°C and optimally at a pH of 5.5. So far, no biochemical study on TACA was reported in literature.

As TA, the bacteria *Sulfurihydrogenibium sp.* (Ssp) belongs to the Aquificales order. Ssp was isolated from the Calcite Hot Springs in Yellowstone National Park (USA) and like TA, grows in 60°C to 80°C temperature range (REF-SSp below). *Sulfurihydrogenibium yellowstonense sp.* nov., an extremely thermophilic, facultatively heterotrophic, sulfur-oxidizing bacterium from Yellowstone National Park, and emended descriptions of the genus *Sulfurihydrogenibium, Sulfurihydrogenibium subterraneum* and *Sulfurihydrogenibium azorense* are described in Nakagawa S, Shtaih Z, Banta A, Beveridge TJ, Sako Y, Reysenbach AL. International Journal of Systematic and Evolutionary Microbiology, 2005 Nov; 55(Pt 6):2263-8. (PubMed ID 16280480).

Distinctly, SspCA grows optimally at pH 7.5, a value two order of magnitude higher than that of TA. Ssp genome contains a gene encoding for an alpha-class carbonic anhydrase hereafter referred as SspCA. Some recent biochemical characterizations of SspCA are reported in literature. However, it is hard to expect TACA properties based on those of SspCA. When comparing TACA polypeptide sequence to all reported protein sequences, SspCA has only 49% sequence identity and 374 other sequences have higher similarity level.

Both SspCA and TACA are believed to be secreted after being produced because of the presence of a signal peptide. In that context, TACA and SspCA have to deal with conditions occurring outside the bacteria. Because of the different optimal growth pH of Ssp vs TA, one could expect SspCA to be more robust than TACA when dissolved in CO_2 capture solvents, the latter being alkaline with pH ranging from 8 to 11. However, the present invention provides results revealing that TACA stability is surprisingly much higher than that of SspCA in tested CO_2 capture solvents.

Referring to Fig 1, an amino acid sequence of a TACA is illustrated. The cleaved signal peptide is underscored and may be replaced with a methionine (SEQ ID NO:4). Various TACA variants and functional derivatives may also be used in the CO_2 capture techniques described herein. For example, the first five amino acids of TACA were replaced by three other amino acids (Fig 16, SEQ ID 6). This change was performed in order to increase enzyme production level and have no impact on TACA stability (Figs 3 to 6).

Referring now to Fig 11, an example of the overall CO_2 capture system 10 includes a source 12 of CO_2 containing gas 14. The source may be a power plant, an aluminum smelter, refinery or another type of CO_2 producing operation at high or atmospheric pressure, or may also be ambient air for some specific applications such as air fractionation or air cleaning. The CO_2 containing gas 14 is supplied to an absorption unit 16, which is also fed with an aqueous absorption solution 18 for contacting the CO_2 containing gas 14. In some implementations, the aqueous absorption solution 18 includes carbonic anhydrase including TACA or a functional derivative thereof and an absorption compound. The carbonic anhydrase may be free in the aqueous absorption solution 18 as dissolved enzyme or aggregates or particles of enzymes. The carbonic anhydrase may be on or in particles that are present in the aqueous absorption solution 18 and flow with it through the absorption unit 16. The carbonic anhydrase may be immobilized with respect to the particles using any method while keeping at least some of its activity. Some immobilization techniques include covalent bonding, entrapment, and so on. The carbonic anhydrase may be immobilized with respect to supports, which may be various structures such as packing material, within the absorption unit 16 so as to remain within the absorption unit 16 as the aqueous absorption solution 18 flows through it.

The CO_2 containing gas 14 may be a CO_2-containing effluent from various sources that includes a proportion of CO_2 and other gases. For example the gas may include from about 0.03% to 60% (v/v) of CO_2 although the CO_2 concentration may be greater. The CO_2-containing gas may also be a gas having high CO_2 content up to 100%, which may be useful for the production of compounds such as sodium bicarbonate from CO_2 gas as one of the starting materials.

The absorption unit 16 may be of various types, such as a packed reactor, a spray reactor, a bubble column type reactor, and so on. There may be one or more reactors that may be provided in series or in parallel. In the absorption unit 16, the TACA catalyses the hydration reaction of CO_2 into bicarbonate and hydrogen ions and thus a CO_2 depleted gas 20 and an ion rich solution 22 are produced.

The ion rich solution 22 is then supplied to a desorption unit 26 to produce a CO_2 stream 28 and an ion depleted solution 30. TACA may also be present to catalyse the dehydration reaction of bicarbonate ions into CO_2 and thus a CO_2 depleted gas 20 and an ion depleted solution 30 is produced. Alternatively, the ion rich solution 22 may be supplied to another type of regeneration step such as mineral carbonation and the like.

Referring now to Fig 12, the system 10 may also include a separation unit 32 arranged in between the absorption unit 16 and the desorption unit 26, for removing at least some and possibly all of the TACA in the event the enzyme is flowing with the ion rich solution 22, e.g. when the enzyme is free in solution or immobilized with respect to particles. The separation unit 32 produces an enzyme depleted stream 34 that may be supplied to the desorption unit 26 and an enzyme rich stream 36 that may be recycled, in whole or in part, to the absorption unit 16. The separation unit may also include one or more separators in series or parallel. The separators may be filters or other types of separators, depending on the removal characteristics for the enzymes and the form of the enzymes or particles.

The system may also include various other treatment units for preparing the ion rich solution 22 for the desorption unit 26 and/or for preparing the ion depleted solution 30 for recycling into the absorption unit 16. There may be pH adjustment units or various monitoring units.

In some implementations, at least some TACA is provided in the desorption unit 26. The TACA may be provided within the input ion-rich solution and/or added separately. The TACA may be tailored, designed, immobilised or otherwise delivered in order to withstand the conditions in the desorption unit 26. TACA may catalyze the conversion of bicarbonate ion to CO_2 as described in Reaction 1 (reverse reaction).

Referring still to Fig 12, the system may also include a measurement device 40 for monitoring properties of various streams and adjusting operation of the absorption unit 16 to

achieve desired properties. Adjusting could be done by various methods including modifying the liquid and/or gas flow rates, for example, or adjusting other operating conditions.

In some implementations, the absorption unit may be operated at conditions so as to leverage the activity and/or stability of the TACA used to catalyze the CO_2 hydration reaction. For example, it has been found that TACA can present high residual activity over a range of elevated temperatures in aqueous absorption solutions including sodium carbonate or potassium carbonate. TACA also presents high activity at lower ambient temperature to provide elevated CO_2 flux in aqueous absorption solutions including sodium carbonate, potassium carbonate or alkanolamines such as MDEA. The operating conditions may include an operating temperature and at least one operating absorption compound within the absorption solution. The operating conditions may further include pH, CO_2 loading, gas and liquid flow rates and compositions, and so on.

In some implementations, the operating conditions are coordinated for maximum leverage of the TACA functionality in CO_2 capture.

In some implementations, the operating conditions may include temperature conditions that, depending on various other parameters of the CO_2 capture operation, may provide an absorption temperature higher than 10°C and lower than 98°C, such as between 25 and 80°C, 30 and 70°C or 40 and 50°C or such as 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, 95°C, 98°C, or any temperature in between. It should also be understood that the temperature conditions in the absorption unit may vary within a certain temperature range, since the operating temperatures at different locations within the absorption unit will be different. In addition, the temperature of the absorption solution can substantially fluctuate throughout absorption and desorption stages that can be used in some CO_2 capture operations.

In some implementations, the operating conditions may include pressure conditions that, depending on various other parameters of the CO_2 capture operation, may provide an absorption pressure higher than 1 bar and lower than 100 bar, such as 2 bars, 5 bars, 10 bars, 20 bars, 25 bars, 30 bars, 35 bars, 40 bars, 45 bars, 50 bars, 55 bars, 60 bars, 65 bars, 70 bars, 75 bars, 80 bars, 85 bars, 90 bars, 95 bars, 100 bars, or any pressure in between.

In some implementations, the operating conditions may include temperature conditions that, depending on various other parameters of the CO_2 capture operation, may provide a desorption temperature higher than 10°C and lower than 110°C, such as between 30 and 110°C, 35 and 90°C or 40 and 70°C or such as 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, 95°C, 100°C, 105°C, 110°C or any temperature in between. It should also be understood that the temperature conditions in the desorption unit may vary within a certain temperature range, since the operating temperatures at different locations within the desorption unit will be different. In addition, the temperature of the absorption solution can substantially fluctuate throughout absorption and desorption stages that can be used in some CO_2 capture operations.

In some implementations, the operating conditions may include pressure conditions that, depending on various other parameters of the CO_2 capture operation, may provide a desorption pressure higher than 0.05 bar and lower than 50 bar, such as 0.1 bar, 0.2 bars, 0.3 bar, 0.4 bar, 0.5 bar, 0.6 bar, 0.7 bar, 0.8 bar, 0.9 bar, 1 bar, 2 bars, 5 bars, 10 bars, 15 bars, 20 bars, 25 bars, 30 bars, 35 bars, 40 bars, 45 bars, 50 bars or any pressure in between.

In some implementations, the operating conditions may include an aqueous absorption solution including an absorption compound, which will be further discussed below.

The enzyme is preferably used in combination with an absorption solution that will supply the CO_2 carrying capacity for the process. The solution may have a composition allowing acceleration of the enzyme catalytic rate by capturing the hydrogen ion released during the hydration reaction. Using TACA allows the CO_2 capture operation to be accelerated, reducing the size of the required capture vessels and associated capital costs. In addition, by taking advantage of this accelerative mechanism, energetically favorable absorption compounds such as tertiary and hindered amines, carbonate/bicarbonate solutions and amino acids/amino acid salts can be employed to reduce associated process energy consumption, where these absorption compounds would normally be too slow to be used efficiently without enzymatic catalysis.

The aqueous absorption solution may include at least one absorption compound that aids in the absorption of CO_2. The absorption compound may include potassium carbonate, sodium

carbonate, ammonium carbonate, at least one amine, which may be a primary amine, a secondary amine, a tertiary amine, a primary alkanolamine, a secondary alkanolamine, a tertiary alkanolamine, and/or an amino acid with primary, secondary or tertiary amino group(s) or a combination thereof. Combinations of absorption compounds include a carbonate and at least one of the amines and/or amino acids mentioned therein or herein, to produce a promoted carbonate absorption solution.

In some scenarios, the absorption compound may include monoethanolamine (MEA), 2-amino-2-methyl-1-propanol (AMP), 2-(2-aminoethylamino)ethanol (AEE), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris or AHPD), N-methyldiethanolamine (MDEA), dimethylmonoethanolamine (DMMEA), diethylmonoethanolamine (DEMEA), triisopropanolamine (TIPA), triethanolamine (TEA), DEA, DIPA, MMEA, TIA, TBEE, HEP, AHPD, hindered diamine (HDA), bis-(tertiarybutylaminoethoxy)-ethane (BTEE), ethoxyethoxyethanol-tertiarybutylamine (EEETB), bis-(tertiarybutylaminoethyl)ether, 1,2-bis-(tertiarybutylaminoethoxy)ethane and/or bis-(2-isopropylaminopropyl)ether, and the like.

In some scenarios, the absorption compound may include piperidine, piperazine, derivatives of piperidine, piperazine which are substituted by at least one alkanol group, dialkylether of polyalkylene glycols, dialkylether or dimethylether of polyethylene glycol, amino acids comprising glycine, proline, arginine, histidine, lysine, aspartic acid, glutamic acid, methionine, serine, threonine, glutamine, cysteine, asparagine, valine, leucine, isoleucine, alanine, tyrosine, tryptophan, phenylalanine, and derivatives such as taurine, N,cyclohexyl 1,3-propanediamine, N-secondary butyl glycine, N-methyl N-secondary butyl glycine, diethylglycine, dimethylglycine, sarcosine, methyl taurine, methyl-α-aminopropionicacid, N-(β-ethoxy)taurine, N-(β-aminoethyl)taurine, N-methyl alanine, 6-aminohexanoic acid, potassium or sodium salt of the amino acid or a combination thereof.

The absorption compound used to make up the aqueous absorption solution may be at least one of the example compounds, i.e. potassium carbonate, sodium carbonate and/or MDEA.

In some scenarios, the concentration of the absorption compound in the solution may be between about 0.1 M and about 10 M, depending on various factors. When the absorption compound is amine-based, the concentration of the amine-based solution may be between about 0.1M and 8M and when the absorption compound is amino acid-based, the

concentration of the amino acid-based solution may be between about 0.1M and 6M. When the absorption compound is carbonate based, the pH of the absorption solution may be between about 8 and about 12, depending for example on the absorption compound and on the CO_2 loading of the solution.

The TACA may be dissolved in the absorption solution. The concentration of the TACA or functional derivative thereof may be between about 0.1 and about 50 g/L, between about 0.01 and about 10 g/L or between about 0.1 and about 5 g/L. When the TACA is not dissolved in the solution but is rather immobilized on mobile particles or fixed packing material, the amount of immobilized TACA may be similar so as to provide a similar activity as the therein mentioned concentrations of dissolved TACA.

As noted above, the TACA or functional derivative thereof may be provided free or dissolved in the solvent, immobilized or entrapped or otherwise attached to particles that are in the absorption solution or to packing material or other structures that are fixed within the reaction chamber.

In the case where the TACA or functional derivative thereof is immobilized with respect to a support material, this may be accomplished by an immobilization technique selected from adsorption, covalent bonding, entrapment, copolymerization, cross-linking, and encapsulation, or combination thereof.

In one scenario, the TACA or functional derivative thereof may be immobilized on a support that is in the form of particles, beads or packing. Such supports may be solid or porous with or without coating(s) on their surface. The TACA or functional derivative thereof may be covalently attached to the support and/or the coating of the support, or entrapped inside the support or the coating. The coating may be a porous material that entraps the TACA or functional derivative thereof within pores and/or immobilizes the TACA by covalent bonding to the surfaces of the support. The support material may be made from a compound different than the TACA or functional derivative thereof. The support material may include nylon, cellulose, silica, silica gel, chitosan, polyacrylamide, polyurethane, alginate, polystyrene, polymethylmetacrylate, magnetic material, sepharose, titanium dioxide, zirconium dioxide and/or alumina, respective derivatives thereof, and/or other materials. The support material may have a density between about 0.6 g/ml and about 5 g/ml such as a

density above 1g/ml, a density above 2 g/mL, a density above 3 g/mL or a density of about 4 g/mL.

In some scenarios, the TACA or functional derivative thereof may be provided as cross-linked enzyme aggregates (CLEAs) and/or as cross-linked enzyme crystals (CLECs).

In the case of using enzymatic TACA particles, including CLEAs or CLECs, the particles may be sized to have a diameter at or below about 17 µm, optionally about 10 µm, about 5 µm, about 4 µm, about 3 µm, about 2 µm, about 1 µm, about 0.9 µm, about 0.8 µm, about 0.7 µm, about 0.6 µm, about 0.5 µm, about 0.4 µm, about 0.3 µm, about 0.2 µm, about 0.1 µm, about 0.05 µm, or about 0.025 µm. The particles may also have a distribution of different sizes.

The TACA used in connection with the techniques described herein may be an isolated and/or substantially pure form.

There is also provided a carbonic anhydrase polypeptide or functional derivatives thereof, which is stable and active at a broad range of temperatures.

In one aspect, the invention provides a carbonic anhydrase polypeptide comprising the sequence as set forth in SEQ ID NO 2, 4 or 6 or functional derivative thereof, an expression or cloning vector comprising a nucleotide sequence encoding such carbonic anhydrase, and a transgenic cell comprising such expression or cloning vector.

The TACA or the derivative thereof can be used in various processes and scenarios such as those described in the following patent references: CA 2.291.785; CA 2.329.113, CA 2.393.016, CA 2,443,222, US 6,908,507; EP 1 377 531, US 7,514,056, US 7,596,952; US 8,066,965, US 8,277,769, US 6,946,288, US 7,740,689, WO2012/103653, US 2013/0203155, CA 2,769,771, US 2012/0122195, US 8,722,391, CA 2,554,395, CA 2,738,061, WO2014/066999.

DEFINITIONS

In order to further appreciate some of the terms used herein, the following definitions and discussion are provided.

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The expression "polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers, and to longer chains generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids, optionally polypeptides may contain glycine, proline, arginine, histidine, lysine, aspartic acid, glutamic acid, methionine, serine, threonine, glutamine, cysteine, asparagine, valine, leucine, isoleucine, alanine, tyrosine, tryptophan, phenylalanine, selenocysteine, selenomethionine, pyrrolysine. "Polypeptide(s)" include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide.

The expression "functional derivative" refers to a protein/peptide/polypeptide sequence that possesses a functional biological activity that is substantially similar to the biological activity of the original protein/peptide/polypeptide sequence. In other words, it refers to a polypeptide of the carbonic anhydrase as defined herein that substantially retain(s) the capacity of catalyzing the hydration of carbon dioxide. A functional derivative of the carbonic anhydrase protein/peptide as defined herein may or may not contain post-translational modifications such as covalently linked carbohydrates, if such modifications are not necessary for the performance of a specific function. The "functional derivative" may also comprise nucleic acid sequence variants encoding the protein/peptide/polypeptide of the invention. These variants may result from the degeneracy of the genetic code or from a mutation, substitution, addition or deletion. Further, the carbonic anhydrase as defined herein may comprise a Tag such as a histidine Tag. The term "functional derivative" is meant to encompass the "variants", the "mutants", the "fragments" or the "chemical derivatives" of a carbonic anhydrase protein/peptide. Methods for measuring carbonic anhydrase activity are known such as stirred cell reactor assay or the method described by Chirica et al. (Chirica et al. European Journal of Biochemistry, 1997, 244, 755-60). These functional derivatives have at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% 99% or 99.5% identity with the sequence

as set forth in SEQ ID NO 2, 4, 6, optionally over the entire length of the sequence or on a partial alignment of the sequences.

The term "polynucleotide fragment", as used herein, refers to a polynucleotide whose sequence (e.g., cDNA) is an isolated portion of the subject nucleic acid constructed artificially (e.g., by chemical synthesis) or by cleaving a natural product into multiple pieces, using restriction endonucleases or mechanical shearing, or a portion of a nucleic acid synthesized by PCR, DNA polymerase or any other polymerizing technique well known in the art, or expressed in a host cell by recombinant nucleic acid technology well known to one of skill in the art.

The term "polypeptide or fragments thereof" as used herein refers to peptides, oligopeptides and proteins. This term also does not exclude post-expression modification of polypeptides. For example, polypeptides that include the covalent attachment of glycosyl groups, acetyl groups, lipid groups and the like are encompassed by the term polypeptide.

Techniques for determining nucleic acid and amino acid "sequence identity" are known in the art. Typically, such techniques include determining the nucleotide sequence of the mRNA for a gene and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequence and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, Nucl. Acids Res. 14(6):6745-6763 (1986). An exemplary implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, Wis.)

in the "BestFit" utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, Wis.). Another method of establishing percent identity which can be used in the context of the present invention is the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, Calif.). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+Swiss protein+Spupdate+PIR.

By "substantially identical" when referring to a polypeptide, it will be understood that the polypeptide of the present invention preferably has an amino acid sequence having at least about 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84% 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99,5% or any other value in between to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6, or functional derivatives thereof, optionally over the entire length of the peptide.

One can use a program such as the CLUSTAL program to compare amino acid sequences. This program compares amino acid sequences and finds the optimal alignment by inserting spaces in either sequence as appropriate. It is possible to calculate amino acid identity or homology for an optimal alignment. A program like BLASTp will align the longest stretch of similar sequences and assign a value to the fit. It is thus possible to obtain a comparison where several regions of similarity are found, each having a different score. Both types of identity analysis are contemplated for the present invention.

With respect to protein or polypeptide, the term "isolated polypeptide" or "isolated and purified polypeptide" is sometimes used herein. This term refers primarily to a protein produced by expression of an isolated and modified polynucleotide molecule contemplated by the invention. Alternatively, this term may refer to a protein which has been sufficiently separated from other proteins with which it would naturally be associated, so as to exist in "substantially pure" form.

The term "substantially pure" refers to a preparation comprising at least 50 % by weight of the carbonic anhydrase polypeptide or derivative thereof on total protein content. More preferably, the preparation comprises at least 75% by weight, and most preferably 90-99% by weight, of the carbonic anhydrase polypeptide or derivative thereof.

Purity is measured by methods appropriate for the carbonic anhydrase polypeptide or derivative thereof as described herein (e.g. chromatographic methods, agarose or polyacrylamide gel electrophoresis, HPLC analysis, and the like).

The TACA polypeptide or TACA functional derivative thereof may also comprise amino acids substitution such that the carbonic anhydrase or TACA functional derivative thereof retains catalytic activity (i.e. the interconversion of CO_2 with HCO_3^- and H^+). The term "substituted amino acid" is intended to include natural amino acids and non-natural amino acids. Non-natural amino acids include amino acid derivatives, analogues and mimetics. As used herein, a "derivative" of an amino acid refers to a form of the amino acid in which one or more reactive groups on the compound have been derivatized with a substituent group. As used herein an "analogue" of an amino acid refers to a compound that retains chemical structures of the amino acid necessary for functional activity of the amino acid yet also contains certain chemical structures that differ from the amino acid. As used herein, a "mimetic" of an amino acid refers to a compound in that mimics the chemical conformation of the amino acid.

As used herein, the term "polynucleotide(s)" generally refers to any polyribonucleotide or poly-deoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. This definition includes, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, cDNA, single- and double-stranded RNA, and RNA that is a mixture of single- and

double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded regions. The term "polynucleotide(s)" also embraces short nucleotides or fragments, often referred to as "oligonucleotides", that due to mutagenesis are not 100% identical but nevertheless code for the same amino acid sequence.

By "substantially identical" when referring to a polynucleotide, it will be understood that the polynucleotide of the invention has a nucleic acid sequence which encodes a polypeptide which is at least about 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84% 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99,5% or any other value between 60 and 99,5% identical to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID 6 or functional derivative thereof.

By "substantially identical" when referring to a polynucleotide, it will be understood that the polynucleotide of the invention has a nucleic acid sequence which is at least about 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84% 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99,5% or any other value between 60 and 99,5% identical to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or functional derivative thereof.

With reference to polynucleotides of the invention, the term "isolated polynucleotide" is sometimes used. This term, when applied to DNA, refers to a DNA molecule that is separated from sequences with which it is immediately contiguous to (in the 5' and 3' directions) in the naturally occurring genome of the organism from which it was derived. For example, the "isolated polynucleotide" may comprise a DNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a procaryote or eucaryote. An "isolated polynucleotide molecule" may also comprise a cDNA molecule.

As used herein, the term "vector" refers to a polynucleotide construct designed for transduction/transfection of one or more cell types. Vectors may be, for example, cloning vectors which are designed for isolation, propagation and replication of inserted nucleotides, expression vectors which are designed for transcription of a nucleotide sequence in a host cell, or a viral vector which is designed to result in the production of a recombinant virus or virus-like particle, or shuttle vectors, which comprise the attributes of more than one type of vector. A number of vectors suitable for stable transfection of cells and bacteria are available

to the public (e.g. plasmids, adenoviruses, baculoviruses, yeast baculoviruses, plant viruses, adeno-associated viruses, retroviruses, Herpes Simplex Viruses, Alphaviruses, Lentiviruses), as are methods for constructing such cell lines. It will be understood that the present invention encompasses any type of vector comprising any of the polynucleotide molecules of the invention.

The term "transgenic cell" refers to a genetically engineered cell. Methods for genetically engineering a cell are known such as molecular cloning and gene targeting. These methods can include chemical-based transfection, non chemical method, particle-based method or viral method. The host cell may be any type of cell such as a transiently-transfected or stably-transfected mammalian cell line, an isolated primary cell, an insect cell, a yeast (*Saccharomyces cerevisiae* or *Pichia pastoris*), a plant cell, a microorganism, or a bacterium (such as *E. coli*).

The expressions "naturally occurring" or" wild-type" refer to material in the form as it occurs in nature. For example, a naturally occurring or wild-type polypeptide or polynucleotide sequence is a sequence present in an organism that is isolated from a source in nature and which has not been intentionally modified by human manipulation.The expressions "Recombinant","engineered" or "non-naturally occurring" : it do not appears in nature, it is an artificial construct. e.g., a cell, nucleic acid, or polypeptide, refers to a material that either has been modified in a manner that would not otherwise be found in nature, or is identical thereto but produced or derived from synthetic materials and/or by manipulation using recombinant techniques.

The expression "Reference sequence" refers to a defined sequence to which another sequence is compared. In one aspect of the invention, the reference sequence is SEQ ID NO 2 and preferably SEQ ID NO 4.

The expression "Reference enzyme" is a known enzyme, such as the TACA enzyme or the SspCA enzyme. The activity of the enzyme of the invention is compared to the activity of a reference enzyme.

The expression "Coding sequence" refers to the nucleic acid sequence(s) that would yield the amino acid sequence of a given protein/peptide/polypeptide.

The term "Non-conservative substitution" refers to an amino acid, at a given position in a protein sequence that is different and not similar from the one in the reference sequence.

The term "Deletion" refers to one or several amino acid(s) at a given position in a protein sequence, that is or are absent when compared to the reference sequence.

The term "Insertion" refers to one or several amino acid(s) at a given position in a protein sequence, that is or are in excess when compared to the reference sequence.

The term "Improved enzyme property" refers to a property that is better in one enzyme when compared to the reference one. It can be an increase in stability toward some denaturing agent, an increase in thermostability, an increase in solvent stability, an increase in pH stability, an increase in enzyme activity, reduced inhibition by products (eg. bicarbonate and/or carbonate ions), improved stability in presence of the sodium cation, improved stability in presence of the potassium cation, improved solvent solubility, an increase in hydrophilicity, an increase in hydrophobicity or a combination thereof.

The term "Stability in presence of" refers to the capacity of the enzyme to remain active over a period of time when in the presence of a denaturing compound. It is usually described as a percentage of remaining activity over time.

The term "Thermostability" refers to the capacity of the enzyme to remain active over a period of time when when exposed to a given temperature. It is usually described as a percentage of remaining activity over time.

The term "Solvent stability" refers to the capacity of the enzyme to remain active over a period of time when when exposed to a given solvant. It is usually described as a percentage of remaining activity over time.

The term "pH stability" refers to the capacity of the enzyme to remain active over a period of time when when exposed to a given pH, such as a higher pH. It is usually described as a percentage of remaining activity over time.

The term "Increased enzyme activity" refers to the capacity of an enzyme to catalyze more reaction, such as hydration of CO_2 and/or dehydratation of the HCO_3^- ion, per time unit than

the reference enzyme in some given conditions, such as higher Temperature, higher pH (improved pH activity profile).

The term "increase hydrophilicity" refers to the property of the enzyme to be more soluble in water based absorption solution.

The term "increase hydrophobicity refers to the property of the enzyme to be less soluble in water based absorption solution.

By "about", it is meant that the relevant value (e.g. of temperature, concentration, pH, etc.) can vary within a certain range depending on the margin of error of the method or apparatus used to evaluate such value. For instance, the margin of error of the temperature may range between ± 0.5°C to ± 1°C, the margin of error of the pH may be ± 0.1 and the margin of error of the concentration may be ± 20%.

In some implementations, TACA can be used in a CO2 capture operation where the absorption and desorption stage are run within certain temperature conditions to leverage TACA's temperature and solvent stability. For example, the absorption stage can be operated between 40°C and 60°C and the desorption stage can be operated between 40°C and 70°C. The absorption and desorption stages can also be configured such that the TACA flows through each stage and has residence times within each stage that further leverage TACA's temperature and solvent stability. For example, the residence time in the absorption stage can be 1 minute to 10 minutes and the residence time in the desorption stage can be 1 minute to 10 minutes. In addition, the concentration of the TACA in the solution can be provided such that catalytic activity is promoted for enhanced residual activity in the CO2 capture process. For example, the TACA can be provided in sufficiently high concentration so as to maintain near 100% residual activity through at least 14 days of operation.

The tests show that TACA was better than all other tested enzymes between 60 and98 C after a certain amount of time. Since TACA is stable, it maintains 100% residual activity over all temperatures for at least 1 hour; when used at 2 or 4 g/L, the residual activity is higher compared to 1 g/L especially after 14 days. Activity determinations are conducted so there is no over-saturation with enzyme.

As TACA has been found to have higher residual activity than all of the comparative carbonic anhydrases that were tested, as illustrated in the examples section, TACA can be used in a CO_2 capture operation with greater efficiency and performance compared to other carbonic anhydrases.

In some implementations, a TACA variant can have a sequence facilitating production, such that the TACA can be used for top-up and replenishing enzymatically enhanced CO_2 capture operations. The TACA top-up frequency and amount can be provided such that high catalysis is maintained.

Various aspects of the present invention will be more readily understood by referring to the following examples. These examples are illustrative of the wide range of applicability of the present invention and are not intended to limit its scope. Modifications and variations can be made therein without departing from the spirit and scope of the invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred methods and materials are described.

The scope of the claims should not be limited by the aspects, scenarios, implementations, examples or embodiments set forth in the examples and the description, but should be given the broadest interpretation consistent with the description as a whole.

The issued patents, published patent applications, and references that are mentioned herein are hereby incorporated by reference. In the case of inconsistencies, the present disclosure will prevail.

EXAMPLES

Example 1: Materials, methods and producing of TACA having a polypeptide sequence described in SEQ ID NO 4

A TACA enzyme was produced without the signal peptide: the first 20 amino acids were replaced by a single methionine. The first 20 amino acids (signal peptide) are underlined in Fig 1 (SEQ ID NO 2). The enzyme was purified and characterized in CO_2 capture columm

and by a pH indicator-based technique. The resulting coding nucleotide sequence is shown in Fig 13 (SEQ ID NO 3) and the encoded TACA amino acid sequence is shown at Fig 14 (SEQ ID NO 4). Amino acid residue numbering will follow that of Fig 14 (SEQ ID NO 4).

The CO_2 capture column consists in contacting a gas containing 14% v/v CO_2 and a CO_2-capture solvent consisting of 1.45M $KHCO_3/K_2CO_3$ pH 10 at 25°C. When present, the enzyme is dissolved in the solvent at a concentration of 0.2g/L. The solvent flows inside a 50 cm height packed column from top to the bottom. The CO_2-containing gas flows countercurrently inside the same column. The Liquid to gas flowrate ratio is adjusted to 50 g/g . A gas analyzer measures the CO_2 concentration in the gas at the inlet and outlet of the column.

The pH indicator-based technique was performed to compare the stability and activity of TACA with those of other carbonic anhydrases. TACA was compared with the following other carbonic anhydrases:

(i) Carbonic anhydrase from *Sulfurihydrogenibium sp referred as "SspCA"* (SEQ ID NO 7) and described in patent application WO2014066999 A1 while having 49 % identity with SEQ ID NO 4 and

(ii) A thermostable variant of the *Sullfurihydrogenibium sp carbonic anhydrase (SspCA)* referred to as "6M1" (SEQ ID NO 8), described in patent application WO2014066999 A1 (SEQ ID NO 196) and having 50% identifty with SEQ ID NO 4 .

Example 2: Performance of TACA in a packed column absorption unit.

An experiment was conducted in an absorption packed column. The absorption solution is an aqueous solution of potassium carbonate 1.45 M at pH 10. This absorption solution is contacted counter-currently with a gas phase with a CO_2 concentration of 130,000 ppm. Liquid flow rate was 500 g/min and gas flow rate was 10 g/min corresponding to L/G of 50 g/g. Gas and absorption solution were at room temperature. The column has a 7.5 cm diameter and a 50 cm height. Packing material is polymeric Raschig™ rings 6 mm. The TACA concentration was 0.2 g/L. The results showed that CO_2 transfer rate of CO_2 removal rate increased from 4.7 mmole/sec for the solution to 40 mmole/sec when adding the

enzyme to the absorption solution. TACA increased the CO_2 removal rate by 8.5 fold under these conditions.

Example 3: Stability of TACA compared to that of SspcA and 6M1

The stability of TACA, SspCA and 6M1 enzymes were compared. The stability was evaluated by exposing the enzymes to an absorption solution including 1.45M $KHCO_3/K_2CO_3$ (2.9M K^+) pH 10 and 20% w/v MDEA alpha=0.1 at various temperatures for different exposure times. As shown in Figs 3 to 10, in all tested conditions, TACA exhibited the highest stability.

As shown in Fig 4, in 1.45M $KHCO_3/K_2CO_3$ (2.9M K^+) pH 10, TACA retains all its activity after one week incubation at 60°C while other tested enzymes have lost more than 60% of their initial activity. In the same way, TACA shows 50% residual activity level after 60 hours incubation at 75°C while other enzyme returned 10% or less residual activity levels (Fig 5). TACA is also the best enzyme at higher temperatures (see Figs 3, 6, 7).

In 20% MDEA alpha=0.1, TACA shows 100% of its initial activity after 28 days incubation at 60°C (Fig 10). During the same time, SspCA is inactivated while 6M1 still exhibits some activity.

Example 4. Stability of TACA compared to that SspcA and 6M1 in the context of thermal cycling in 1.45M $KHCO_3/K_2CO_3$ (2.9M K^+) pH 10

In industrial application, enzymes will have to deal with temperature fluctuations. To test the enzyme stability in this context, a thermal cycling test was conducted on TACA. The enzyme was subjected to temperature fluctuations occurring between 30°C and 75°C. Fig 9 shows temperature profile occurring for each cycle which lasts about 8 minutes. This cycle was repeated 180 times per day for 28 days, giving a total of 5040 cycles. Under these conditions, TACA retained about 50-100% residual activity level after 7-14 days. About 25-50% activity level was recorded after 28 days.

Example 5: Comparison of amino acid sequences between carbonic anhydrase obtained from Thermovibrio ammonificans and the most similar protein in GenBank

As shown at Fig 2, the most similar carbonic anhydrase to the carbonic anhydrase obtained from *Thermovibrio ammonificans* is from *Persephonella marina* with 66% identity. SspCA, not shown in Fig 2, is ranked as the 375th most similar protein.

CLAIMS

1. A method for absorbing CO_2 from a CO_2-containing gas, comprising:

   contacting the CO_2-containing gas with an aqueous absorption solution to dissolve the CO_2 into the aqueous absorption solution; and

   providing a *Thermovibrio ammonificans* carbonic anhydrase (TACA) or functional derivative thereof to catalyze the hydration reaction of the dissolved CO_2 into bicarbonate and hydrogen ions.

2. The method of claim 1, wherein the method comprises providing operating conditions such that the TACA displays enhanced stability and/or activity compared to a reference enzyme.

3. The method of claim 1, wherein the TACA provides an enhanced CO_2 flux of at least 8.5 times a corresponding CO_2 flux with no enzyme.

4. The method of any one of claims 1 to 3, wherein the aqueous absorption solution comprises at least one absorption compound.

5. The method of claim 4, wherein the at least one absorption compound comprises a primary amine, a secondary amine, a tertiary amine, a primary alkanolamine, a secondary alkanolamine, a tertiary alkanolamine, a primary amino acid, a secondary amino acid, a tertiary amino acid, dialkylether of polyalkylene glycols, dialkylether or dimethylether of polyethylene glycol, amino acid or a derivative thereof, monoethanolamine (MEA), 2-amino-2-methyl-1-propanol (AMP), 2-(2-aminoethylamino)ethanol (AEE), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris or AHPD), N-methyldiethanolamine (MDEA), dimethylmonoethanolamine (DMMEA), diethylmonoethanolamine (DEMEA), triisopropanolamine (TIPA), triethanolamine (TEA), DEA, DIPA, MMEA, TIA, TBEE, HEP, AHPD, hindered diamine (HDA), bis-(tertiarybutylaminoethoxy)-ethane (BTEE), ethoxyethoxyethanol-tertiarybutylamine (EEETB), bis-(tertiarybutylaminoethyl)ether, 1,2-bis-(tertiarybutylaminoethoxy)ethane and/or bis-(2-isopropylaminopropyl)ether, or a combination thereof.

6. The method of claim 4, wherein the at least one absorption compound comprises a primary amine, a secondary amine, a tertiary amine, a primary alkanolamine, a secondary alkanolamine, a tertiary alkanolamine, a primary amino acid, a secondary amino acid, a tertiary amino acid or a combination thereof.

7. The method of claim 4, wherein the at least one absorption compound comprises dialkylether of polyalkylene glycols, dialkylether or dimethylether of polyethylene glycol, amino acid or derivative thereof or a combination thereof.

8. The method of claim 4, wherein the at least one absorption compound comprises piperazine or derivative thereof.

9. The method of claim 8, wherein the piperazine or derivatives thereof are substituted by at least one alkanol group.

10. The method of claim 4, wherein the at least one absorption compound comprises monoethanolamine (MEA), 2-amino-2-methyl-1-propanol (AMP), 2-(2-aminoethylamino)ethanol (AEE), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris or AHPD), N-methyldiethanolamine (MDEA), dimethylmonoethanolamine (DMMEA), diethylmonoethanolamine (DEMEA), triisopropanolamine (TIPA), triethanolamine (TEA), DEA, DIPA, MMEA, TIA, TBEE, HEP, AHPD, hindered diamine (HDA), bis-(tertiarybutylaminoethoxy)-ethane (BTEE), ethoxyethoxyethanol-tertiarybutylamine (EEETB), bis-(tertiarybutylaminoethyl)ether, 1,2-bis-(tertiarybutylaminoethoxy)ethane and/or bis-(2-isopropylaminopropyl)ether.

11. The method of claim 4, wherein the at least one absorption compound comprises an amino acid or derivative thereof.

12. The method of claim 11, wherein the amino acid or derivative thereof comprises glycine, proline, arginine, histidine, lysine, aspartic acid, glutamic acid, methionine, serine, threonine, glutamine, cysteine, asparagine, valine, leucine, isoleucine, alanine, tyrosine, tryptophan, phenylalanine, taurine, N,cyclohexyl 1,3-propanediamine, N-secondary butyl glycine, N-methyl N-secondary butyl glycine,diethylglycine, dimethylglycine, sarcosine, methyl taurine, methyl-α-aminopropionicacid, N-(β-ethoxy)taurine, N-(β-aminoethyl)taurine, N-methyl

alanine, 6-aminohexanoic acid, potassium or sodium salt of the amino acid or a combination thereof.

13. The method of claim 4, wherein the absorption compound comprises a carbonate compound.

14. The method of claim 4, wherein the absorption compound comprises sodium carbonate, potassium carbonate or MDEA.

15. The method of claim 4, wherein the absorption compound comprises sodium carbonate.

16. The method of claim 4, wherein the absorption compound comprises potassium carbonate.

17. The method of any one of claims 1 to 16, wherein the temperature of the absorption solution is at least 10°C.

18. The method of any one of claims 1 to 16, wherein the temperature of the absorption solution is at least 25°C.

19. The method of any one of claims 1 to 18, wherein the step of contacting is performed at a temperature between about 10°C and about 98°C.

20. The method of any one of claims 1 to 18, wherein the step of contacting is performed at a temperature between about 25°C and about 80°C.

21. The method of any one of claim 1 to 18, wherein the step of contacting is performed at a temperature between about 30°C and about 70°C.

22. The method of any one of claim 1 to 18, wherein the step of contacting is performed at a temperature between about 40°C and about 50°C.

23. The method of any one of claims 1 to 22, wherein the concentration of the TACA or functional derivative is between about 0.01 g/L and about 50 g/L in the absorption solution, optionally between about 0.3g/L and about 10g/L.

24. The method of any one of claims 1 to 23, wherein the pH of the absorption solution is between about 8 and about 11.

25. The method of any one of claims 1 to 24, wherein the CO_2 loading is between about 0.05 and about 1 mol CO_2/mol amine or mol CO_2/mol cation.

26. The method of any one of claims 1 to 25, further comprising subjecting the ion-rich solution to desorption to produce a regenerated absorption solution and a CO_2 gas stream.

27. The method of any one of claims 1 to 26, wherein at least a portion of the TACA or functional derivative is a component of the absorption solution and the ion-rich solution and catalyzes the desorption reaction.

28. The method of any one of claims 1 to 27, wherein the absorption is operated at a temperature between about 10°C and about 98°C, optionally between about 25°C and about 80°C, between about 30^0C and about 70^0C, or between about 40°C and about 50°C, optionally at 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80 °C, 85°C, 90°C, 95°C or 98°C or any other value in between.

29. The method of any one of claims 1 to 28, wherein the desorption is operated at a temperature between about 30^0C and about 110^0C, optionally between about 35^0C and about 90^0C or between about 40^0C and about 70^0C.

30. A method for CO_2 capture, comprising:

in an absorption stage:

contacting a CO_2-containing gas with an aqueous absorption solution to dissolve the CO_2 into the aqueous absorption solution;

providing *Thermovibrio ammonificans* carbonic anhydrase (TACA) or functional derivative thereof in the absorption solution to catalyze the hydration reaction of the dissolved CO_2 into bicarbonate and hydrogen ions, thereby producing an ion-rich solution comprising at least some of the TACA and a CO_2-depleted gas; and/or

in a desorption stage:

providing conditions for treating the ion-rich solution comprising at least some of the TACA or functional derivative, so as to desorb CO_2 gas from the ion-rich solution, thereby producing a regenerated absorption solution and a CO_2 gas stream.

31. The method of claim 30, wherein the absorption stage is operated with the following absorption operating parameters:

absorption temperature in between about 10°C and about 98°C;

concentration of an absorption compound in the absorption solution between about 0.1M and about 5M;

pH of the absorption solution in between about 8 and about 11; and/or

CO_2 loading in between about 0.05 and about 1 mol CO_2/mol amine or mol CO_2/mol cation.

32. The method of claim 30 or 31, wherein the desorption stage is operated with the following desorption operating parameter:

desorption temperature in between about 30°C and about 110°C.

33. The method of any one of claims 30 to 32, wherein the absorption stage and desorption stage are operated within an overall operating temperature zone wherein the TACA or functional derivative shows 100% residual activity after at least 1 week of exposure to overall operating temperature zone.

34. The method of any one of claims 30 to 33, wherein the absorption stage and desorption stage are operated within an overall operating temperature zone wherein the TACA or functional derivative provides enhanced temperature stability compared to a reference enzyme.

35. A method for desorption of CO_2 from a solution comprising bicarbonate and hydrogen ions, comprising providing conditions desorption of the CO_2 in the presence of a *Thermovibrio ammonificans* carbonic anhydrase (TACA) or functional derivative thereof, so as to catalyze

the desorption of CO_2 gas from the solution, thereby producing an ion-depleted solution and a CO_2 gas stream.

36. A system for absorbing CO_2 from a CO_2-containing gas, comprising:

an absorption unit comprising:

a gas inlet for receiving the CO_2-containing gas;

a liquid inlet for receiving an aqueous absorption solution;

a reaction chamber for contacting the CO_2-containing gas with the aqueous absorption solution to dissolve the CO_2 into the aqueous absorption solution, wherein *Thermovibrio ammonificans* carbonic anhydrase (TACA) or functional derivative thereof is present for catalyzing the hydration reaction of the dissolved CO_2 into bicarbonate and hydrogen ions, thereby producing an ion-rich solution and a CO_2-depleted gas;

a liquid outlet for releasing the ion-rich solution; and

a gas outlet for releasing the CO_2-depleted gas.

37. The system of claim 36, further comprising a regeneration stage for regenerating the ion-rich solution.

38. The system of claim 37, wherein the regeneration stage comprises a desorption unit and/or a mineralization unit.

39. The system of any one of claims 36 to 38 further comprising a temperature regulator for regulating the temperature of the absorption unit to promote enhanced stability of the TACA or functional derivative thereof.

40. The method of any one of claims 34 to 36, wherein the operating conditions are provided such that the combined stability and activity of the TACA or functional derivative provide enhanced overall CO_2 capture over time per given enzyme utilization.

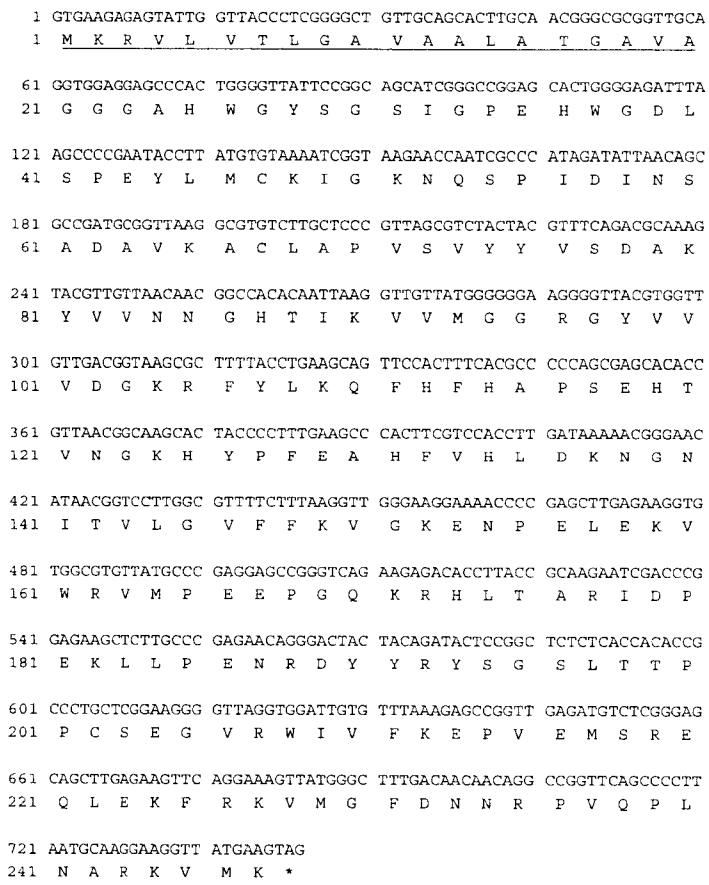


Figure 1

Genbank accession number	Description	Query cover	Ident
WP_013538320.1	carbonic anhydrase [Thermovibrio ammonificans]	98%	100%
WP_015898908.1	carbonic anhydrase [Persephonella marina]	98%	66%
WP_029522463.1	carbonic anhydrase [Persephonella sp. KM09-Lau-8]	98%	63%
WP_029521561.1	carbonic anhydrase [Persephonella sp. IF05-L8]	98%	61%
WP_007474387.1	carbonic anhydrase [Caminibacter mediatlanticus]	98%	59%
WP_028579713.1	hypothetical protein [Desulfobulbus japonicus]	98%	52%
WP_019445033.1	carbonic anhydrase [Aeromonas sp. 159]	98%	53%
WP_007040788.1	carbonic anhydrase [Thiorhodococcus drewsii]	98%	52%
WP_005354260.1	carbonic anhydrase [Aeromonas veronii]	98%	53%
WP_005362587.1	carbonic anhydrase [Aeromonas veronii]	98%	53%
WP_005348316.1	carbonic anhydrase [Aeromonas veronii]	98%	53%
WP_007766615.1	Carbonic anhydrase [Cronobacter turicensis]	100%	49%

Figure 2

CA 02890582 2015-05-05

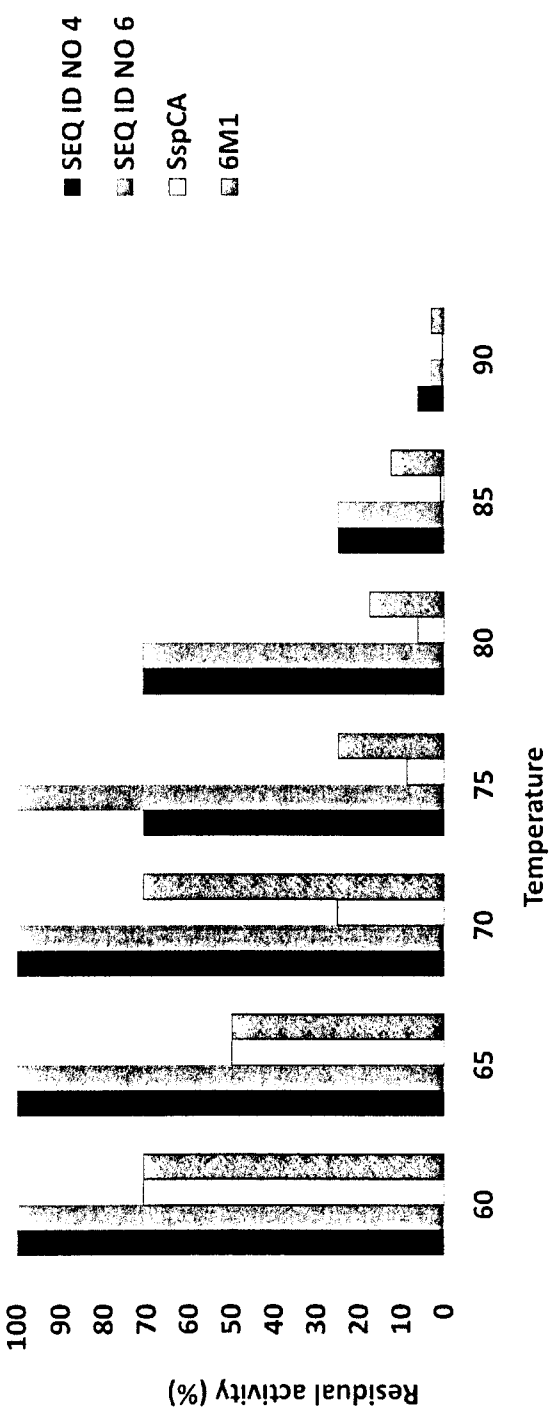

SEQ ID NO 4
SEQ ID NO 6
SspCA
6M1
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
60
65
70
75
80
85
90
Temperature

Figure 3

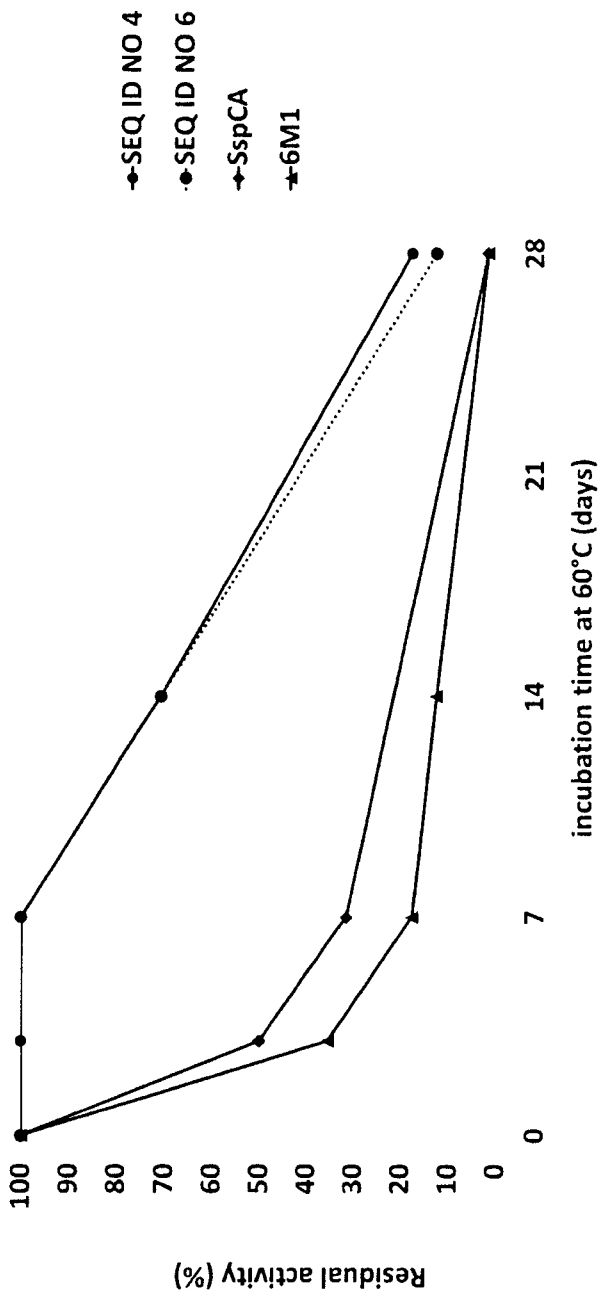

SEQ ID NO 4
SEQ ID NO 6
SspCA
6M1
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
7
14
21
28
incubation time at 60°C (days)

Figure 4

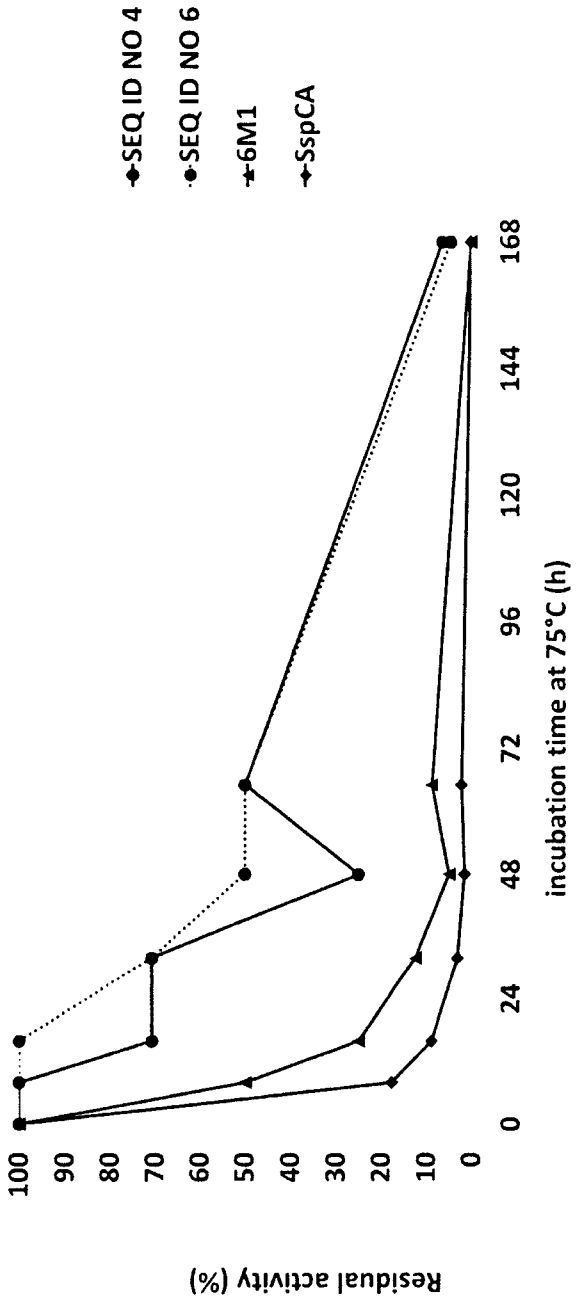
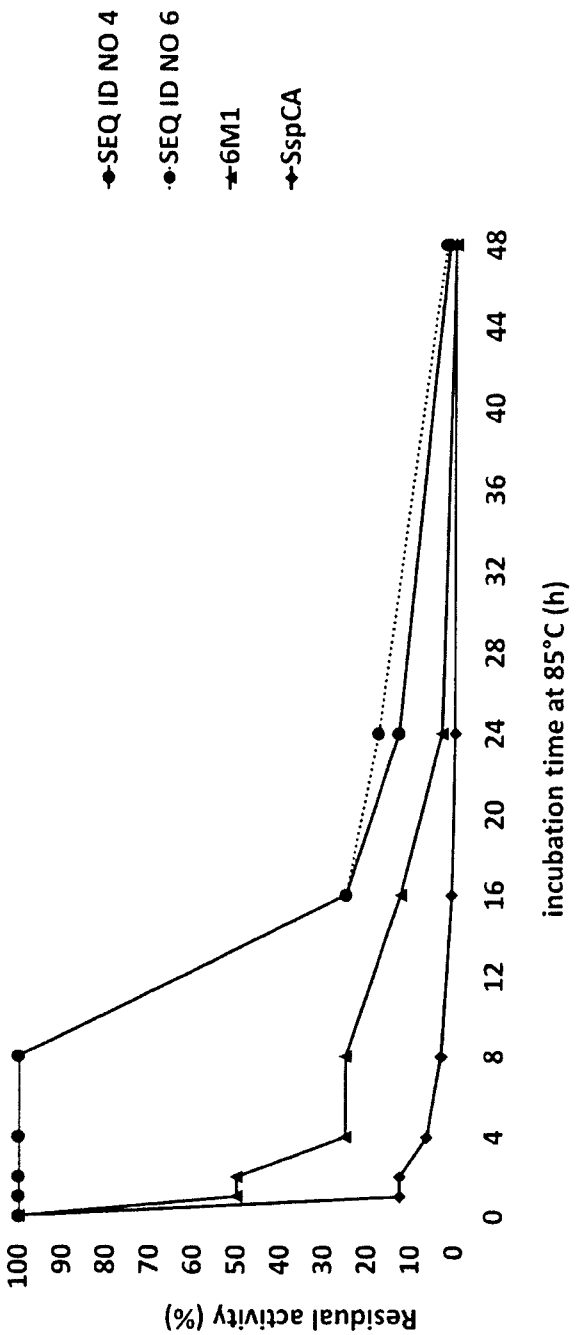

SEQ ID NO 4
SEQ ID NO 6
6M1
SspCA
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120
144
168
incubation time at 75°C (h)

Figure 5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SEQ ID NO 6
6M1
SspCA
Residual activity (%)
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90
80
70
60
50
40
30
20
10
0
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4
8
12
16
20
24
28
32
36
40
44
48
incubation time at 85°C (h)

Figure 6

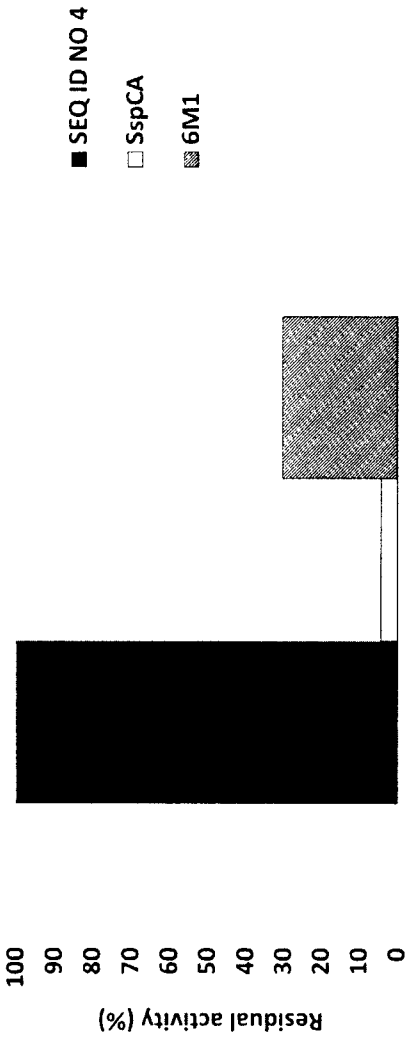
SEQ ID NO 4
SspCA
6M1
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0

Figure 7

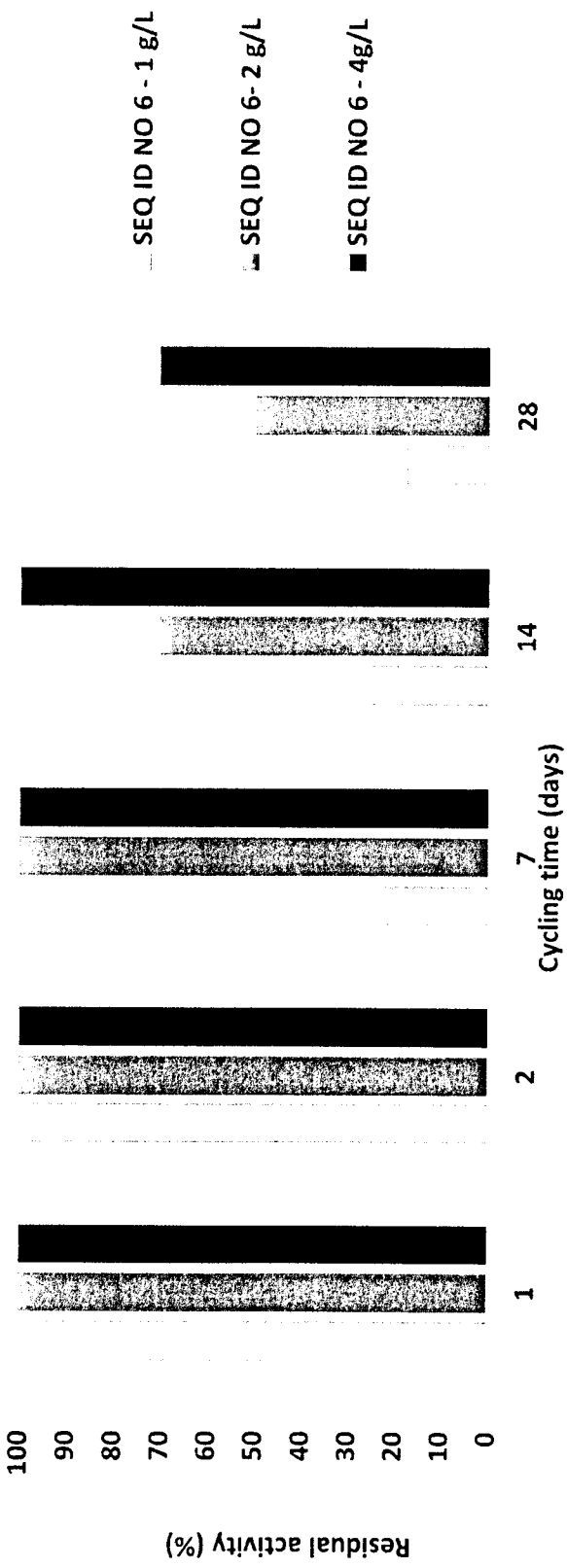
SEQ ID NO 6 - 1 g/L
SEQ ID NO 6- 2 g/L
SEQ ID NO 6 - 4g/L
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
7
14
28
Cycling time (days)

Figure 8


Temperature (°C)
85
75
65
55
45
35
25
Time (minute:second)
00:00
00:20
00:40
01:00
01:20
01:40
02:00
02:20
02:40
03:00
03:20
03:40
04:00
04:20
04:40
05:00
05:20
05:40
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06:40
07:00
07:20
07:40
08:00

Figure 9

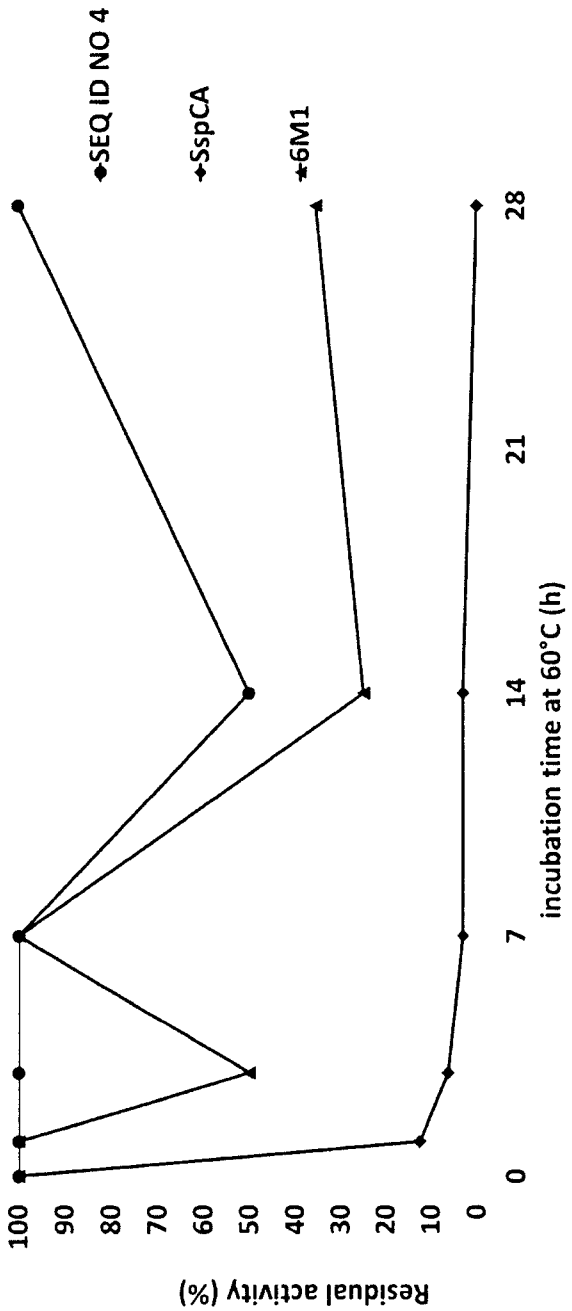
SEQ ID NO 4
SspCA
6M1
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
7
14
21
28
incubation time at 60°C (h)

Figure 10

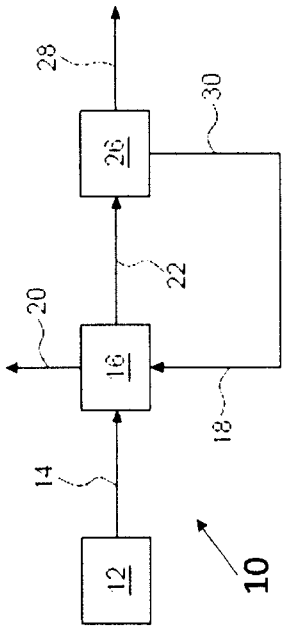

Figure 11

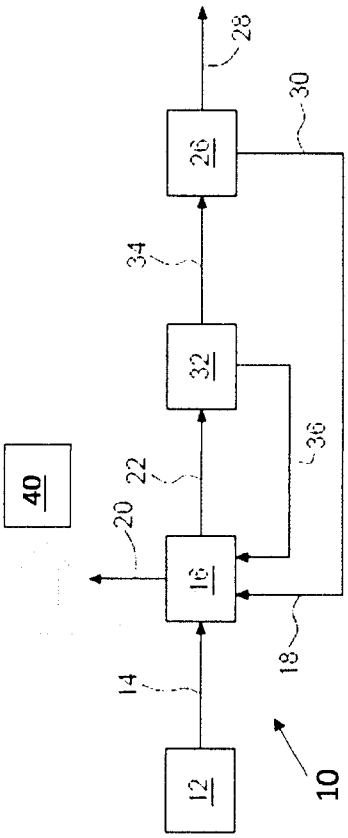

Figure 12

```
  1 ATGGGTGGCGGTGCACATTGGGGTTATAGCGGTTCGATTGGTCCAGAACATTGGGGTGAC
 61 TTGTCCCCGGAGTACCTGATGTGTAAAATCGGTAAGAATCAATCCCCGATTGATATTAAT
121 AGCGCGGACGCGGTTAAGGCATGCCTGGCACCAGTTAGCGTCTACTATGTCAGCGATGCC
181 AAATACGTTGTGAACAACGGCCATACCATTAAAGTTGTGATGGGCGGTCGTGGTTATGTC
241 GTCGTTGATGGCAAACGTTTCTACCTGAAACAGTTCCACTTCCACGCGCCGAGCGAGCAC
301 ACGGTTAACGGCAAGCACTACCCGTTCGAGGCTCACTTTGTGCACCTGGATAAGAATGGT
361 AATATCACCGTTCTGGGCGTGTTTTTCAAGGTTGGCAAGGAAAATCCGGAGCTGGAAAAA
421 GTGTGGCGCGTTATGCCGGAAGAACCGGGCCAGAAGCGTCATTTGACCGCCCGTATCGAC
481 CCTGAGAAGCTGCTGCCGGAAAACCGCGACTATTACCGTTATTCTGGTAGCCTGACGACT
541 CCGCCGTGCAGCGAGGGTGTCCGTTGGATCGTCTTTAAAGAGCCGGTGGAGATGAGCCGC
601 GAACAACTGGAGAAATTTCGTAAAGTGATGGGTTTTGACAACAACCGTCCGGTGCAGCCG
661 CTGAATGCGCGCAAAGTCATGAAGTAA
```

Figure 13
(SEQ ID NO 3)

Figure 14
(SEQ ID NO 4)

```
  1 ATGGAACACGAATGGGGTTATAGCGGTTCGATTGGTCCAGAACATTGGGGTGACTTGTCC
 61 CCGGAGTACCTGATGTGTAAAATCGGTAAGAATCAATCCCCGATTGATATTAATAGCGCG
121 GACGCGGTTAAGGCATGCCTGGCACCAGTTAGCGTCTACTATGTCAGCGATGCCAAATAC
181 GTTGTGAACAACGGCCATACCATTAAAGTTGTGATGGGCGGTCGTGGTTATGTCGTCGTT
241 GATGGCAAACGTTTCTACCTGAAACAGTTCCACTTCCACGCGCCGAGCGAGCACACGGTT
301 AACGGCAAGCACTACCCGTTCGAGGCTCACTTTGTGCACCTGGATAAGAATGGTAATATC
361 ACCGTTCTGGGCGTGTTTTTCAAGGTTGGCAAGGAAAATCCGGAGCTGGAAAAAGTGTGG
421 CGCGTTATGCCGGAAGAACCGGGCCAGAAGCGTCATTTGACCGCCCGTATCGACCCTGAG
481 AAGCTGCTGCCGGAAAACCGCGACTATTACCGTTATTCTGGTAGCCTGACGACTCCGCCG
541 TGCAGCGAGGGTGTCCGTTGGATCGTCTTTAAAGAGCCGGTGGAGATGAGCCGCGAACAA
601 CTGGAGAAATTTCGTAAAGTGATGGGTTTTGACAACAACCGTCCGGTGCAGCCGCTGAAT
661 GCGCGCAAAGTCATGAAGTAA
```

Figure 15
(SEQ ID NO 5)

Figure 16
(SEQ ID NO 6)