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(73) Patenthaver: **Saipem S.p.A., Via Luigi Russolo, 5, 20138 Milano, Italien**

(72) Opfinder: **VOYER, Normand, , 20138 MILANO, Italien**
DAIGLE, Richard, , 20138 MILANO, Italien
MADORE, Éric, , 20138 MILANO, Italien
FRADETTE, Sylvie, , 20138 MILANO, Italien

(74) Fuldmægtig i Danmark: **Zacco Denmark A/S, Arne Jacobsens Allé 15, 2300 København S, Danmark**

(54) Benævnelse: **Fremgangsmåde og system til opsamling af CO2 ved hjælp af Thermovibrio ammonificans-kulsyreanhydrase**

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WO-A1-2014/066999
JO BYUNG HOON ET AL: "Bacterial extremo-[alpha]-carbonic anhydrases from deep-sea hydrothermal vents as potential biocatalysts for CO2sequestra", JOURNAL OF MOLECULAR CATALYSIS. B, ENZYMATIC, ELSEVIER, AMSTERDAM, NL, vol. 109, 15 August 2014 (2014-08-15), pages 31-39, XP029048191, ISSN: 1381-1177, DOI: 10.1016/J.MOLCATB.2014.08.002
PAUL JAMES ET AL: "The structure of a tetrameric [alpha]-carbonic anhydrase from Thermovibrio ammonificans reveals a core formed around intermolecular disulfides that contribute to its thermostability", ACTA CRYSTALLOGRAPHICA SECTION D BIOLOGICAL CRYSTALLOGRAPHY, vol. 70, no. 10, 27 September 2014 (2014-09-27), pages 2607-2618, XP055464597, DOI: 10.1107/S1399004714016526
JO ET AL.: 'Bacterial extremo-alpha-carbonic anhydrases from deep-sea hydrothermal vents as potential biocatalysts for C02 sequestration".' JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC vol. 109, 15 August 2014, pages 31 - 39, XP029048191 DOI: 10.1016/J.MOLCATB.2014.08.002

DESCRIPTION

TECHNICAL FIELD

[0001] The technical field relates to CO_2 capture and the use of *Thermovibrio ammonificans* carbonic anhydrase (TACA) according to claim 1 and 11 and dependent thereof for catalyzing the hydration reaction of CO_2 into bicarbonate and hydrogen ions and/or catalyzing the desorption reaction to produce a CO_2 gas.

BACKGROUND

[0002] 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.

[0003] 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.

[0004] 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.

[0005] 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, a, R, y, 15 and g. 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:

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

[0006] Under optimum conditions, the catalyzed turnover rate of the hydration reaction can reach 1×10^6 molecules/second.

[0007] 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.

[0008] There is thus a need to overcome at least some of the challenges related to the use of carbonic anhydrase for CO_2 capture.

[0009] The prior-art document WO 2014/066999 discloses the use of a *Thermovibrio ammonificans* carbonic anhydrase (TACA) for the separation of CO_2.

[0010] The article of Jo et al. ("Bacterial extremo-alpha-carbonic anhydrases from deep-sea hydrothermal vents as potential biocatalysts for CO2 sequestration", Journal of Molecular Catalysis B: ENZYMATIC, vol. 109, 15 August 2014) discloses the properties of a carbonic anhydrase obtained from *Persephonella marina* and *Thermovibrio ammonificans* in the processes for the absorption of CO_2.

SUMMARY

[0011] As per claim 1, it is disclosed a method for absorbing CO_2 from a CO_2-containing gas, the method comprising:

contacting the CO_2-containing gas with an aqueous absorption solution to dissolve the CO_2 into the aqueous absorption solution at commercial scale process conditions; and

providing a *Thermovibrio ammonificans* carbonic anhydrase (TACA) variant with at least 70% identity to SEQ ID NO 6 and having residues corresponding to positions 2 to 6 of SEQ ID NO: 4 replaced by Glu-His-Glu, to catalyze the hydration reaction of the dissolved CO_2 into bicarbonate and hydrogen ions,

wherein the aqueous absorption solution comprises at least one absorption compound, and

wherein the concentration of the carbonic anhydrase in the absorption solution is between 0.1 g/L and 5 g/L.

[0012] As per claim 2, in the method the at least one absorption compound comprises a carbonate compound, preferably sodium carbonate or potassium carbonate.

[0013] As per claim 3, in the method of the invention the aqueous absorption solution comprises at least one absorption compound comprising:

1. (a) 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;
2. (b) dialkylether of polyalkylene glycols, dialkylether or dimethylether of polyethylene glycol, amino acid or derivative thereof, or a combination thereof;
3. (c) piperazine or derivative thereof, optionally substituted by at least one alkanol group;
4. (d) monoethanolamine (MEA), 2-amino-2-methyl-1-propanol (AMP), 2-(2-aminoethylamino)ethanol (AEE), 2-amino-2-hydroxymethy1-1,3-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
5. (e) an amino acid or derivative thereof, 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-aminopropionic acid, N-(6-ethoxy)taurine, N-(6-aminoethyl)-taurine, N-methyl alanine, 6-aminohexanoic acid, potassium or sodium salt of the amino acid, or a combination thereof.

[0014] As per claim 4, the pH of the absorption solution is between 8 and 11.

[0015] As per claim 5, the CO_2 loading is between 0.05 and 1 mol CO_2/mol amine or mol CO_2/mol cation.

[0016] As per claim 6, the absorption is operated at a temperature between 10°C and 98°C, optionally between 25°C and 80°C, between 30°C and 70°C, or between 40°C and 50°C.

[0017] As per claim 7, the method of the invention may further comprise subjecting the aqueous absorption solution comprising bicarbonate and hydrogen ions to desorption to produce a regenerated absorption solution and a CO_2 gas stream, wherein at least a portion of the carbonic anhydrase catalyzes the desorption reaction.

[0018] As per claim 8, the desorption is operated at a temperature between 30°C and 110°C, optionally between 35°C and 90°C, or between 40°C and 70°C.

[0019] As per claim 9, the operating conditions comprise a temperature swing between the absorption and the desorption, wherein the temperature swing is between 25°C and 105°C, optionally between 30°C and 85°C, or between 40°C and 60°C.

[0020] As per claim 10, in the method of the invention the carbonic anhydrase has at least 80%, at least 90%, at least 95%, or at least 98% identity with the sequence of SEQ ID NO 6.

[0021] As per claim 11, the invention discloses an enzyme-enhanced CO_2 capture system 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_2into the aqueous absorption solution;

a *Thermovibrio ammonificans* carbonic anhydrase (TACA) variant with at least 70% identity to SEQ ID NO: 6 and having residues corresponding to positions 2 to 6 of SEQ ID NO: 4 replaced by Glu-His-Glu, that 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, wherein the concentration of the carbonic anhydrase in the absorption solution is between 0.1 g/L and 5 g/L;

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

a gas outlet for releasing the CO_2-depleted gas;

a heat exchanger for heating the ion-rich solution to produce a heated ion-rich solution;

a stripper unit comprising:

a liquid inlet for receiving the ion-rich solution;

a stripping chamber for allowing CO_2 to be released from the ion-rich solution to produce CO_2 gas stream and a regenerated solution, wherein the carbonic anhydrase is present for catalyzing the dehydration reaction;

a liquid outlet for releasing the regenerated solution; and

a gas outlet for releasing the CO_2 gas stream; and

a recycle system for recycling at least a portion of the regenerated solution back to the liquid inlet of the absorption unit as at least part of the aqueous absorption solution.

[0022] As per claim 12, the enzyme-enhanced CO_2 capture system of the invention:

1. (a) further comprises a make-up device for providing make-up carbonic anhydrase to the system;
2. (b) the reaction chamber comprises packing material;
3. (c) the stripping chamber comprises packing material;
4. (d) the carbonic anhydrase is free in solution to cyclically flow between the absorption unit and the stripper unit, or the carbonic anhydrase is immobilized on or in particles that are sized, configured and provided in a concentration so as to flow with the absorption solution and the regenerated solution, such that the particles cyclically flow between the absorption unit and the stripper unit; or
5. (e) any combination of (a) to (d).

[0023] As per claim 13, in the enzyme-enhanced CO_2 capture system of the invention the absorption unit comprises a packed reactor, a spray reactor, a bubble column type reactor, or a process intensification (PI) reactor such as a rotating packed bed (RPB).

[0024] As per claim 14, in the enzyme-enhanced CO_2 capture system of the invention, the CO_2-containing gas is biogas, raw petroleum gas, derived from natural gas combustion, or derived from coal combustion.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025]

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 occurring 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.

Fig 17 shows a polypeptide sequence SEQ ID NO 7 corresponding to polypeptide sequence SEQ ID NO 6 without gap ("-") in order to make residue numbering continuous.

Fig 18 is a graph showing residual activity of TACA over time when continuously exposed to a 1.45 M K_2CO_3 pH 10 solution under temperature cycling conditions.

Fig 19 is a graph showing relative CO_2 absorption rate versus enzyme concentration illustrating the impact of adding TACA to a 1.45 M K_2CO_3 solution at different concentrations on the CO_2 absorption rate of the one tonne per day CO_2 capture unit.

Fig 20 shows the polypeptide sequences of carbonic anhydrase from Sulfurihydrogenibium sp, referred to as "SspCA" (SEQ ID NO 7) (top); and a thermostable variant of the Sullfurihydrogenibium sp carbonic anhydrase (SspCA), referred to as "6M1" (SEQ ID NO 8) (bottom).

DETAILED DESCRIPTION

[0026] Various methods and 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.

[0027] 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-Rodriguez I, Hiigler 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.

[0028] So far, biochemical study on TACA has been limited. Jo BH, Seo JH, Cha HJ, Bacterial extremo-a-carbonic anhydrases from deep-sea hydrothermal vents as potential biocatalysts for CO2 sequestration. Journal of Molecular Catalysis B: Enzymatic. 2014, Nov; 109: p. 3139 (hereafter "Jo et al.") and James P, Isupov MN, Sayer C, Saneei V, Berg S, Lioliou M, Kotlar HK, Littlechild JA. The structure of a tetrameric a-carbonic anhydrase from Thermovibrio ammonificans reveals a core formed around intermolecular disulfides that contribute to its thermostability. Acta Crystallogr D Biol Crystallogr. 2014, Oct; 70 (Pt 10):2607-18 (hereafter "James et al."), describe preliminary assessment of TACA relative to other known CA enzymes. These works test and assess TACA in relatively mild conditions, such as low-concentrated buffer (pH of about 8) and low ionic strength. However, relatively different process conditions are present in real industrial CO_2 capture applications, which may include conditions such as high pH (e.g., 9 to 11), thermal cycling (temperature swings ranging from 25C to 105C, for example, when cycling from absorption to stripping), very high ionic strength, shear forces, turbulence, and large gas-liquid interfaces which promote mass transfer (yet can have denaturing effects). In addition, due to the relatively high concentrations of carbonate ions contained in various CO_2 capture solvents, proteins can face solubility issues, as reported for example in Yanjie Zhang and Paul S. Cremer. Chemistry of Hofmeister Anions and Osmolytes. Annu Rev Phys Chem. 2010. 61 :63-83 (hereafter "Zhang & Cremer") which describes that the carbonate ion can be a highly efficient protein precipitator.

[0029] In addition, neither Jo et al. nor James et al. studied wild type TACA. Jo et al. studied TACA with an extra six histidines tag at the C-terminal end. As shown in the 3D structure of TACA described by James et al., TACA's carboxy terminal functional group is implied in the adoption of a tetrameric organization. Jo et al. suggest that TACA is a dimeric enzyme while James et al. describe TACA as a tetramer. Moreover, the James et al. report that TACA properties can greatly differ according to its oligomerisation state. In James et al., the TACA enzyme which was studied had at its N-terminal end a six histidines tag plus the 20-residues secretion signal. The N-terminal region being close to the active site, significant changes in stability and activity may have occurred.

[0030] As will be described further below, signification work, development and testing have been conducted and found that TACA and functional derivatives thereof are operable in the industrial process conditions of a CO_2 capture operation and can provide even greater temperature stability than reported in literature.

[0031] TACA also provides enhanced performance of enzyme-assisted CO_2 capture compared to other CAs, such as *Sulfurihydrogenibium sp.* (Ssp) CA. Like TA, the bacteria 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 80C 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).

[0032] Distinctly, Ssp 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 or predict 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.

[0033] 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, embodiments of the present invention provide results revealing that TACA stability is surprisingly much higher than that of SspCA in tested relevant CO_2 capture solvents and conditions.

[0034] 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). TACA variant having at least 70% identity to SEQ ID NO 6 and having residues corresponding to positions 2 to 6 of SEQ ID NO: 4 replaced by Glu-His-Glu are used in the CO_2 capture techniques described herein, wherein 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).

[0035] 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 the TACA of the invention 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, encapsulation, 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.

[0036] 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.

[0037] The absorption unit 16 (also referred to as an "absorber" herein) may be of various types, such as a packed reactor, a spray reactor, a bubble column type reactor, a rotating packed bed (RPB) or other type of process intensification (PI) 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 catalyzes 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.

[0038] The ion-rich solution 22 is then supplied to a desorption unit 26 (also referred to herein as a "stripper") to produce a CO_2 stream 28 and an ion depleted solution 30. TACA may also be present to catalyze 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. It should be noted that the ion-rich solution 22 may be heated prior to being supplied to the desorption unit 26.

[0039] 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 flowing 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.

[0040] 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.

[0041] 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, immobilized 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).

[0042] 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 measurement device 40 can monitor the activity of the TACA cycled through the CO_2 capture system 10, and this information can be used to determine, calibrate and/or control the addition of make-up TACA into the system.

[0043] In some implementations, the absorption unit 16 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.

[0044] 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 are provided for commercial scale CO_2 capture operations-such as relatively high pH, high ionic strength, high temperature, and so on-ad the TACA of the invention provides high performance for catalysis of the desired reaction(s) in the cyclic system.

[0045] 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.

[0046] 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.

[0047] 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 1100, 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. It should also be noted that the operating conditions may include a temperature swing between the absorption unit and the desorption unit, and the temperature swing may vary between about 25°C and about 105°C, optionally between about 30°C and about 85°C, or between about 40°C and about 60°C, for example. Different temperature swings can be used depending on various operating parameters, such as type of solvent or absorption compound(s) used in the process.

[0048] 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.

[0049] The operating conditions include an aqueous absorption solution including an absorption compound, which will be further discussed below.

[0050] The enzyme is 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.

[0051] The aqueous absorption solution includes at least one absorption compound that aids in the absorption of CO_2. The absorption compound may include potassium carbonate, sodium carbonate, ammonium carbonate, and/or 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. It should also be noted that the absorption solution can include a single absorption compound, such as potassium carbonate. In addition, the

absorption solution can include a main absorption compound, such as potassium carbonate, and also one or more secondary compounds that may include an amine, for example.

[0052] 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.

[0053] 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-aminopropionic acid, 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.

[0054] 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.

[0055] 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.

[0056] The TACA of the invention may be dissolved in the absorption solution. The concentration of the TACA of the invention 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.

[0057] As noted above, the TACA variant of the invention having at least 70% identity to SEQ

ID NO 6 and having residues corresponding to positions 2 to 6 of SEQ ID NO: 4 replaced by Glu-His-Glu 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.

[0058] In the case where the TACA of the invention 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.

[0059] In one scenario, the TACA of the invention 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 of the invention 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 of the invention 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 of the invention. 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/m L.

[0060] In some scenarios, the TACA of the invention may be provided as cross-linked enzyme aggregates (CLEAs) and/or as cross-linked enzyme crystals (CLECs).

[0061] 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 pm, optionally about 10 pm, about 5 pm, about 4 pm, about 3 pm, about 2 pm, about 1 pm, about 0.9 pm, about 0.8 pm, about 0.7 pm, about 0.6 pm, about 0.5 pm, about 0.4 pm, about 0.3 pm, about 0.2 pm, about 0.1 pm, about 0.05 pm, or about 0.025 pm. The particles may also have a distribution of different sizes.

[0062] The TACA of the invention used in connection with the techniques described herein may be an isolated and/or substantially pure form.

[0063] There is also provided a carbonic anhydrase polypeptide or functional derivatives thereof, which is stable and active at a broad range of temperatures.

[0064] In some aspects, the TACA variant having at least 70% identity to SEQ ID NO 6 and having residues corresponding to positions 2 to 6 of SEQ ID NO: 4 replaced by Glu-His-Glu; may be derived from an expression or cloning vector comprising a nucleotide sequence encoding such carbonic anhydrase, or a transgenic cell comprising such expression or cloning vector.

[0065] The TACA variant having at least 70% identity to SEQ ID NO 6 and having residues corresponding to positions 2 to 6 of SEQ ID NO: 4 replaced by Glu-His-Glu can be used in various processes and scenarios such as those described in the following patent references that are hereby incorporated herein by reference: 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, CA 2,886,708.

Definitions

[0066] In order to further appreciate some of the terms used herein, the following definitions and discussion are provided.

[0067] 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.

[0068] 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).

[0069] 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.

[0070] 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.

[0071] 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.

[0072] 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%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 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.

[0073] 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.

[0074] 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.

[0075] 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.

[0076] 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).

[0077] The TACA variant having at least 70% identity to SEQ ID NO 6 and having residues corresponding to positions 2 to 6 of SEQ ID NO: 4 replaced by Glu-His-Glu 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.

[0078] 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.

[0079] 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%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 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.

[0080] 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%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 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.

[0081] With reference to polynucleotides described herein, 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 prokaryote or eukaryote. An "isolated polynucleotide molecule" may also comprise a cDNA molecule.

[0082] 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.

[0083] 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*).

[0084] 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.

[0085] 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.

[0086] 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.

[0087] The expression "Coding sequence" refers to the nucleic acid sequence(s) that would yield the amino acid sequence of a given protein/peptide/polypeptide.

[0088] 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.

[0089] 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.

[0090] 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.

[0091] 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.

[0092] 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.

[0093] The term "Thermostability" refers to the capacity of the enzyme to remain active over a period of time when exposed to a given temperature. It is usually described as a percentage of remaining activity over time.

[0094] The term "Solvent stability" refers to the capacity of the enzyme to remain active over a period of time when exposed to a given solvent. It is usually described as a percentage of remaining activity over time.

[0095] The term "pH stability" refers to the capacity of the enzyme to remain active over a period of time when exposed to a given pH, such as a higher pH. It is usually described as a percentage of remaining activity over time.

[0096] The term "Increased enzyme activity" refers to the capacity of an enzyme to catalyze more reaction, such as hydration of CO_2 and/or dehydration 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).

[0097] The term "increase hydrophilicity" refers to the property of the enzyme to be more soluble in water based absorption solution.

[0098] The term "increase hydrophobicity refers to the property of the enzyme to be less soluble in water based absorption solution.

[0099] 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%.

[0100] In some implementations, the TACA of the invention can be used in a CO_2 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 opera ted between 40°C and 85°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 of the invention in the solution CO_2 be provided such that catalytic activity is promoted for enhanced residual activity in the CO_2 capture process. For example, the TACA of the invention can be provided in sufficiently high concentration so as to maintain near 100% residual activity through at least 14 days of operation.

[0101] The tests show that the TACA of the invention was better than all other tested enzymes between 60 and 98°C after a certain amount of time. Since the TACA of the invention 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.

[0102] As the TACA of the invention 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.

[0103] According to the invention, a TACA variant with at least 70% identity to SEQ ID NO 6 and having residues corresponding to positions 2 to 6 of SEQ ID NO: 4 replaced by Glu-His-Glu has 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.

[0104] In some aspects, the TACA of the invention has an improved property relative to the same property of the polypeptide of SEQ ID NO 4, selected from one or more of improved stability and or activity and or solubility in presence of sodium ion; improved stability and or activity and or solubility in presence of potassium ion; improved stability and or activity and or solubility in presence of carbonate ion; improved stability and or activity and or solubility under high pH conditions; improved stability and or activity and or solubility under high temperature conditions and improved pH-activity profile.

[0105] In addition, the TACA of the invention assessed in tests reported in the present application display enhanced stability compared to other TACAs assessed by James et al., for example. In James et al., a mild HEPES/NaCl buffer was used and the enzyme was exposed to 90°C for one hour, resulting in complete deactivation. In contrast, the TACA of the invention and having structural differences compared to the James et al. enzymes gave enhanced results in terms of enzyme stability.

[0106] Various aspects of the present invention will be more readily understood by referring to the following examples 1-7, which are not according to the invention.

[0107] 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 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.

EXAMPLES & EXPERIMENTATION

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

[0108] 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 column 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).

[0109] 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.

[0110] 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:

1. (i) Carbonic anhydrase from *Sulfurihydrogenibium sp referred as "SspCA" (SEQ* I D NO 7) and described in patent application WO2014066999 A1 while having 49 % identity with SEQ ID NO 4; and
2. (ii) A thermostable variant of the *Sullfunhydrogenibium 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% identity with SEQ ID NO 4.

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

[0111] 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 UG 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

[0112] 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-CO_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.

[0113] 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 teste d 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).

[0114] In 20% MDEA alpha=0.1, TACA shows 100% of its initial activity after 28 days incubation at 60C (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 themal cycling in 1.45M $KHCO_3/K_2CO_3$ (2.9M K^+) pH 10

[0115] 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

[0116] 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 375^{1h} most similar protein.

Example 6: Stability of TACA under temperature cycling conditions

[0117] To confirm the potential of TACA for CO_2 capture operations, its stability was evaluated under temperature cycling conditions to mimic the process conditions to which it would be exposed. 1.2 L of a 1.45 M K_2CO_3 solution at a CO_2 loading of 0.63 (pH 10), containing 2 g/L of TACA enzyme (SEQ ID6), exposed to a 40°C was continuously pumped through a water bath at a temperature of 77°C where its temperature was increased for 4 minutes. Then the solution was pumped back to the reservoir at 40°C. A temperature of 40°C is typical of conditions in an absorption unit and higher temperatures are representative of temperature to be encountered in a desorption unit. The solution was exposed to these temperature cycling conditions on 24h per day and 7d per week basis. At specific exposure times, samples of the solution were withdrawn for activity measurement. CO_2 hydration activity of TACA was measured at 25°C in a 1.45 M K_2CO_3 pH 10 solution, TACA concentration for the assay was 0.2 g/L. Residual activity data for TACA are available at Figure 18. Results show that this enzyme keeps at least 80% of its initial activity for at least 20 days. In the context of an industrial use of this enzyme in a CO_2 capture unit this clearly demonstrates that the enzyme is robust towards industrially relevant operation conditions characterized by salt concentration higher than 0.5 M and alkaline pH. These tests show that TACA has remarkable stability in practical process conditions which are relatively harsh when compared to standard laboratory conditions and native conditions.

Example 7: Cyclic process performance

[0118] The industrial relevance of TACA (SEQ ID 6) was demonstrated in a 1 tonne per day CO_2 capture pilot unit located at the University of North Dakota's Energy & Environmental Research Center (EERC). The CO_2 capture unit included a packed column absorber and a packed column stripper/desorber. The TACA enzyme was used in combination with a 1.45 M K_2CO_3 solution to capture CO_2 from a gas effluent. Two types of gas effluents were tested: one from natural gas combustion and a second from coal combustion. CO_2 concentration in the

flue gas from the natural gas combustion had a concentration of 10% (v/v) and the one coming from coal combustion had a concentration of 14% (v/v). In addition to CO_2, flue gases included CO and NOx. SOx was also present in flue gases coming from coal combustion.

[0119] The packed column absorber was operated at 30°C. The absorption solution containing potassium carbonate and TACA was fed at the top of the absorber. As the solution counter currently contacted the flue gas, it absorbed CO_2 so the pH of the solution went from 10 to 9. In order to strip the CO_2 out of the absorption solution, the CO_2 loaded solution was sent to a stripper where it was heated using a heating medium at a temperature of 85°C. The CO_2 was released from the solution as a concentrated CO_2 stream. The absorption solution, now a CO_2 lean solution, was sent back to the absorber.

[0120] TACA enzyme concentrations were varied from 0.2 to 2 g/L. Results are shown in Figure 19 and indicate that a small enzyme concentration of 2 grams per liter is sufficient to cause an increase in CO_2 capture performance by near five-fold under tested conditions. The enzyme was used in the pilot unit for 7 days, 24 h/day, without any activity decrease, even when contacted with gas contaminants as NOx, CO and SOx, confirming the industrial relevance of TACA for CO_2 capture operations.

REFERENCES CITED IN THE DESCRIPTION

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This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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Patentkrav

1. Fremgangsmåde til at absorbere CO_2 fra en CO_2-holdig gas, hvilken fremgangsmåde omfatter:

at bringe den CO_2-holdige gas i kontakt med en vandig absorptionsopløsning for at opløse CO_2 i den vandige absorptionsopløsning ved procesbetingelser i kommerciel skala; og

at tilvejebringe en *Thermovibrio ammonificans* kulsyreanhydrase (TACA) variant med mindst 70% identitet med SEQ ID NO 6 og med rester svarende til position 2 til 6 i SEQ ID NO: 4 erstattet af Glu-His-Glu, for at katalysere hydreringsreaktionen af den opløste CO_2 til bicarbonat- og hydrogenioner,

hvor den vandige absorptionsopløsning omfatter mindst én absorptionsforbindelse, og

hvor koncentrationen af kulsyreanhydrasen i absorptionsopløsningen er mellem 0,1 g/L og 5 g/L.

2. Fremgangsmåde ifølge krav 1, hvor den mindst ene absorptionsforbindelse omfatter en carbonatforbindelse, fortrinsvis natriumcarbonat eller kaliumcarbonat.

3. Fremgangsmåde ifølge krav 1, hvor den vandige absorptionsopløsning omfatter mindst én absorptionsforbindelse, der omfatter:

(a) en primær amin, en sekundær amin, en tertiær amin, en primær alkanolamin, en sekundær alkanolamin, en tertiær alkanolamin, en primær aminosyre, en sekundær aminosyre, en tertiær aminosyre eller en kombination deraf;

(b) dialkylether af polyalkylenglycoler, dialkylether eller dimethylether af polyethylenglycol, aminosyre eller derivat deraf, eller en kombination deraf;

(c) piperazin eller derivat deraf, eventuelt substitueret med mindst én alkanolgruppe;

(d) monoethanolamin (MEA), 2-amino-2-methyl-1-propanol (AMP), 2-(2-aminoethylamino)ethanol (AEE), 2-amino-2-hydroxymethyl-1-1,3-propandiol

(Tris) eller AHPD), N-methyldiethanolamin (MDEA), dimethylmonoethanolamin (DMMEA), diethylmonoethanolamin (DEMEA), triisopropanolamin (TIPA), triethanolamin (TEA), DEA, DIPA, MMEA, TIA, TBEE, HEP, AHPD, hindret diamin (HDA) bis-(tertiærbutylaminoethoxy)ethan (BTEE), ethoxyethoxy-ethanol-tertiærbutylamin (EEETB), bis-(tertiærbutylaminoethyl)ether, 1,2-bis-(tertiærbutylaminoethoxy)ethan og/eller bis-(2-isopropylaminopropyl)ether; eller

(e) en aminosyre eller et derivat heraf, hvor aminosyren eller derivatet heraf omfatter glycin, prolin, argininin, histidin, lysin, asparaginsyre, glutaminsyre, methionin, serin, threonin, glutamin, cystein, asparagin, valin, leucin, isoleucin, alanin, tyrosin, tryptofan, phenylalanin, taurin, N,cyclohexyl-1,3-propandiamin, N-sekundær-butylglycin, N-methyl-N-sekundær-butylglycin, diethylglycin, dimethylglycin, sarcosin, methyltaurin, methyl-α-aminopropionsyre, N-(6-ethoxy)taurin, N-(6-aminoethyl)-taurin, N-methylalanin, 6-aminohexansyre, kalium- eller natriumsalt af aminosyren eller en kombination heraf.

4. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 3, hvor pH-værdien af absorptionsopløsningen er mellem 8 og 11.

5. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 4, hvor CO_2-belastningen er mellem 0,05 og 1 mol CO_2/mol amin eller mol CO_2/mol kation.

6. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 5, hvor absorptionen drives ved en temperatur mellem 10°C og 98°C, eventuelt mellem 25°C og 80°C, mellem 30°C og 70°C eller mellem 40°C og 50°C.

7. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 6, yderligere omfattende at udsætte den vandige absorptionsopløsning omfattende bicarbonat- og hydrogenioner for desorption for at producere en regenereret absorptions-

opløsning og en CO_2-gasstrøm, hvor mindst en del af kulsyreanhydrasen katalyserer desorptionsreaktionen.

8. Fremgangsmåde ifølge krav 7, hvor desorptionen drives ved en temperatur mellem 30°C og 110°C, eventuelt mellem 35°C og 90°C eller mellem 40°C og 70°C.

9. Fremgangsmåde ifølge krav 7 eller 8, hvor driftsbetingelserne omfatter et temperatursving mellem absorptionen og desorptionen, hvor temperatursvinget er mellem 25°C og 105°C, eventuelt mellem 30°C og 85°C eller mellem 40°C C og 60°C.

10. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 9, hvor kulsyreanhydrasen har mindst 80%, mindst 90%, mindst 95% eller mindst 98% identitet med sekvensen af SEQ ID NO 6.

11. Enzymforstærket CO_2-opsamlingssystem, der omfatter:
en absorptionsenhed omfattende:
et gasindløb til at modtage den CO_2-holdige gas;
et væskeindløb til at modtage en vandig absorptionsopløsning;
et reaktionskammer til at bringe den CO_2-holdige gas i kontakt med den vandige absorptionsopløsning for at opløse CO_2 i den vandige absorptionsopløsning;
en *Thermovibrio ammonificans* kulsyreanhydrase (TACA) variant med mindst 70% identitet med SEQ ID NO 6 og med rester svarende til position 2 til 6 af SEQ ID NO: 4 erstattet af Glu-His-Glu., der er til stede for at katalysere hydreringsreaktionen af den opløste CO_2 til bicarbonat- og hydrogenioner,
hvorved der produceres en ionrig opløsning og en CO_2-depleteret gas, hvor koncentrationen af kulsyreanhydrasen i absorptionsopløsningen er mellem 0,1 g/L og 5 g/L;
et væskeudløb til frigivelse af den ionrige opløsning; og

et gasudtag til frigivelse af den CO_2-depleterede gas;
en varmeveksler til opvarmning af den ionrige opløsning til fremstilling af en opvarmet ionrig opløsning;
en stripperenhed omfattende:
et væskeindløb til modtagelse af den ionrige opløsning;
et stripningskammer til at gøre det muligt for CO_2 at blive frigivet fra den ionrige opløsning for at producere CO_2-gasstrøm og en regenereret opløsning, hvor kulsyreanhydrasen er til stede til at katalysere dehydreringsreaktionen;
et væskeudløb til frigivelse af den regenererede opløsning; og
et gasudtag til frigivelse af CO_2-gasstrømmen; og
et recirkuleringssystem til recirkulering af i det mindste en del af den regenererede opløsning tilbage til væskeindløbet af absorptionsenheden som i det mindste en del af den vandige absorptionsopløsning.

12. Enzymforstærket CO_2-opsamlingssystem ifølge krav 11, hvor:
(a) systemet yderligere omfatter en suppleringsindretning til at tilvejebringe supplerende kulsyreanhydrase til systemet;
(b) reaktionskammeret omfatter pakningsmateriale;
(c) stripningskammeret omfatter pakningsmateriale;
(d) kulsyreanhydrasen er fri i opløsning til cyklisk at strømme mellem absorptionsenheden og stripperenheden, eller kulsyreanhydrasen er immobiliseret på eller i partikler, der er dimensioneret, konfigureret og tilvejebragt i en koncentration, så de flyder med absorptionsopløsningen og den regenererede opløsning, således at partiklerne cyklisk strømmer mellem absorptionsenheden og stripperenheden; eller
(e) en hvilken som helst kombination af (a) til (d).

13. Enzymforstærket CO_2-opsamlingssystem ifølge krav 11 eller 12, hvor absorptionsenheden omfatter en pakket reaktor, en sprayreaktor, en reaktor af boblesøjletypen eller en procesintensiveringsreaktor (PI), såsom et roterende

pakket leje (RPB).

14. Enzymforstærket CO_2-opsamlingssystem ifølge et hvilket som helst af kravene 11 til 13, hvor den CO_2-holdige gas er biogas, rå petroleumsgas, afledt af naturgasforbrænding eller afledt af kulforbrænding.

DRAWINGS

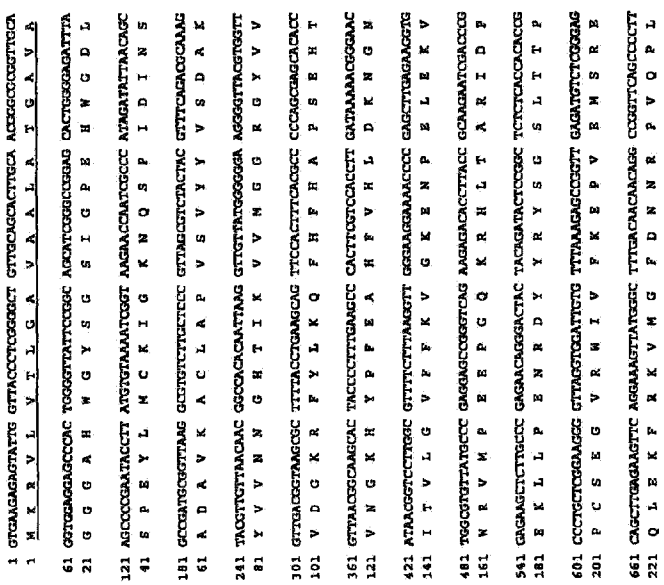


Figure 1

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%
WP_012459296.1	carbonic anhydrase [Sulfurihydrogenibium sp. YO3AOP1 (SspCA)]	97%	49%
not applicable	SspCA variant "6M1" (SEQ ID 9)	97%	50%

Figure 2

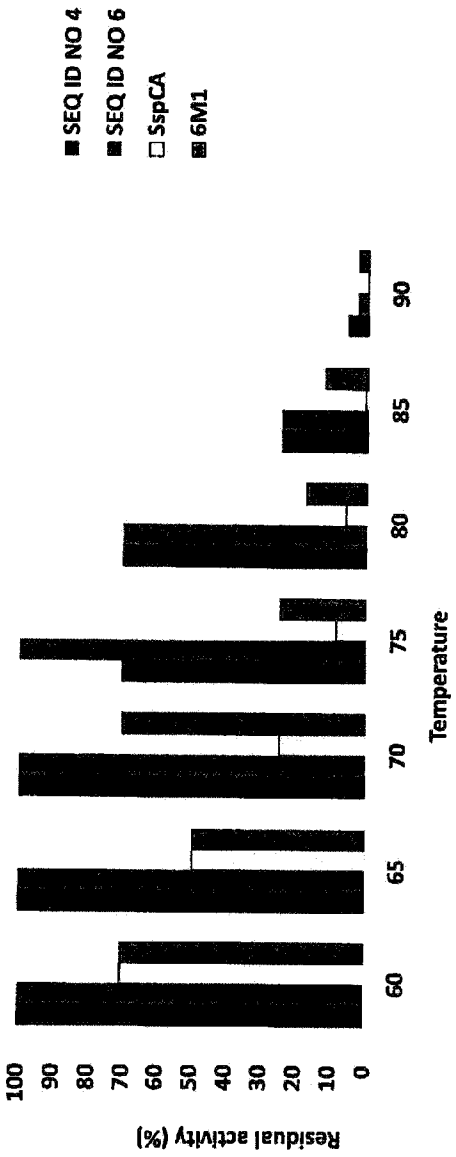

SEQ ID NO 4
SEQ ID NO 6
SspCA
6M1
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
60
65
70
75
80
85
90
Temperature

Figure 3

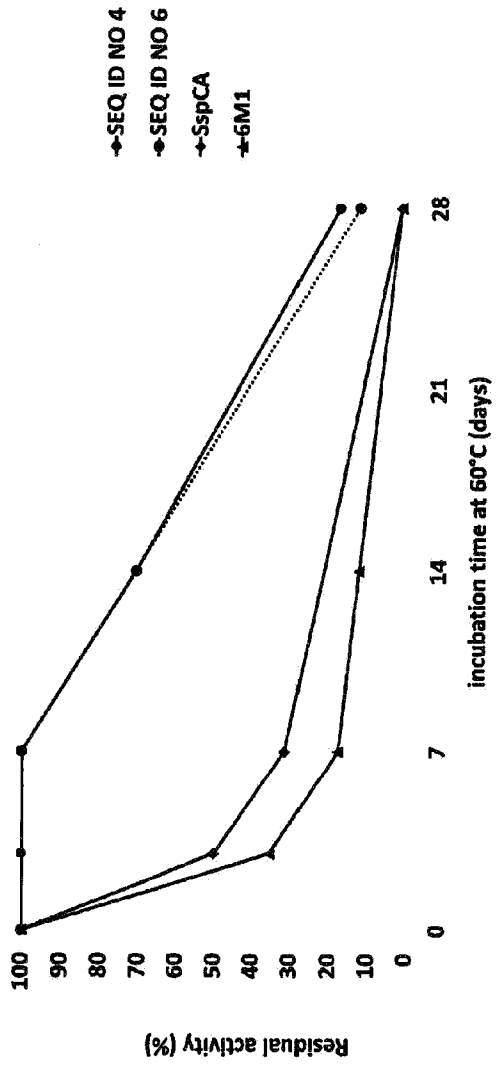

SEQ ID NO 4
SEQ ID NO 6
SspCA
6M1
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
7
14
21
28
incubation time at 60°C (days)

Figure 4

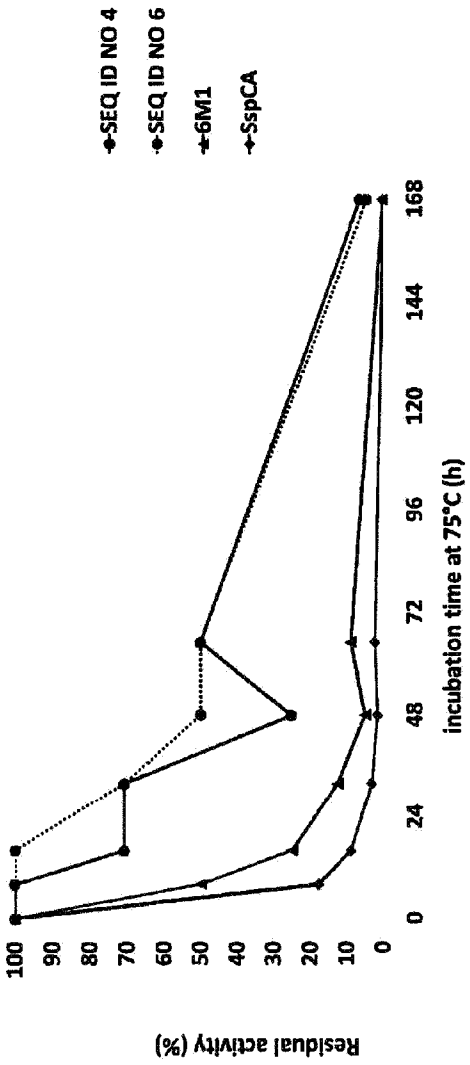

SEQ ID NO 4
SEQ ID NO 6
6M1
SspCA
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120
144
168
incubation time at 75°C (h)

Figure 5

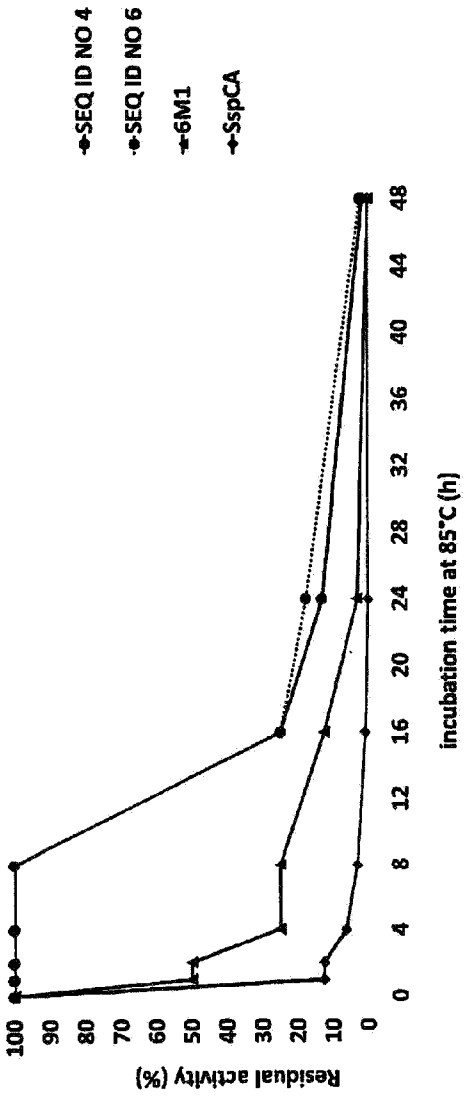
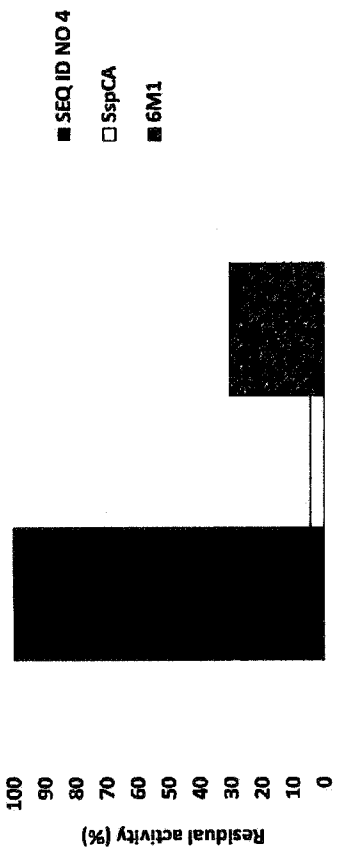

SEQ ID NO 4
SEQ ID NO 6
6M1
SspCA
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
32
36
40
44
48
incubation time at 85°C (h)

Figure 6


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

Figure 7

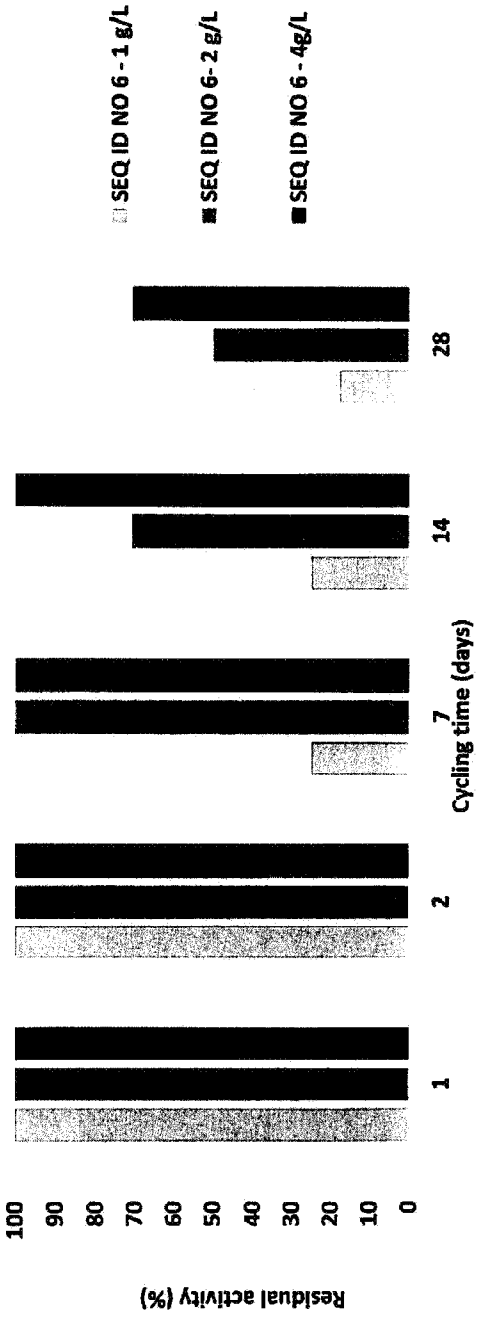
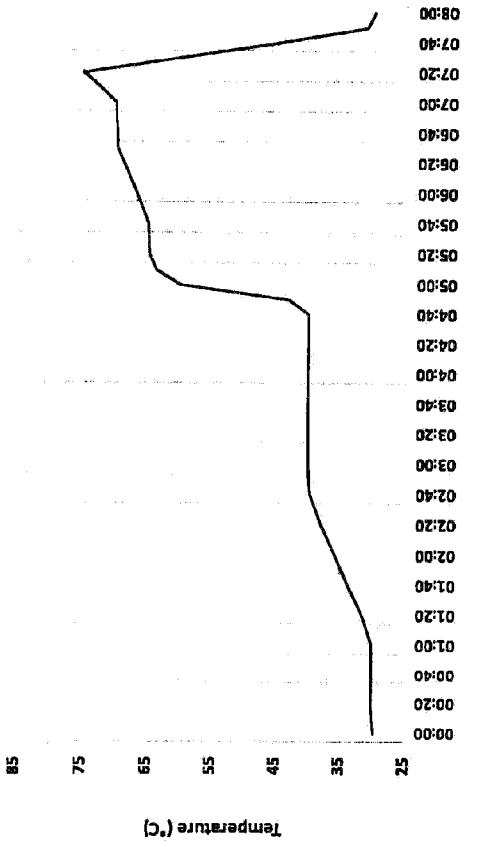

SEQ ID NO 6 - 1 g/L
SEQ ID NO 6- 2 g/L
SEQ ID NO 6 - 4g/L
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
7
14
28
Cycling time (days)

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
06:00
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06:40
07:00
07:20
07:40
08:00

Figure 9

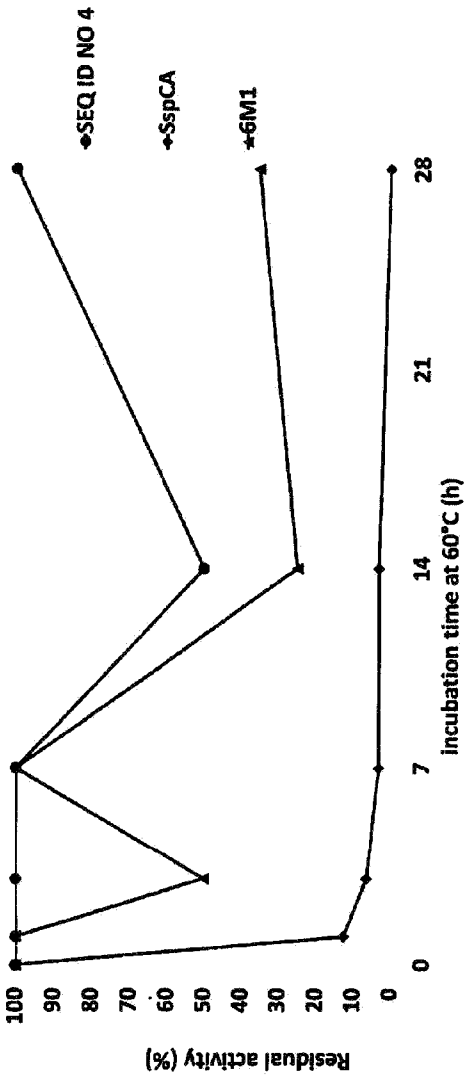
SEQ ID NO 4
SspCA
6M1
Residual activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
7
14
21
28
incubation time at 60°C (h)

Figure 10

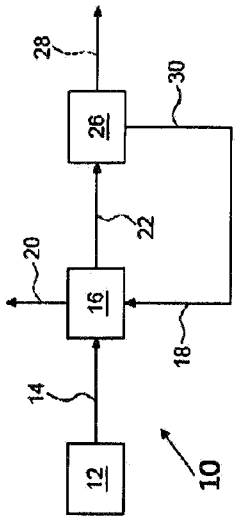

Figure 11

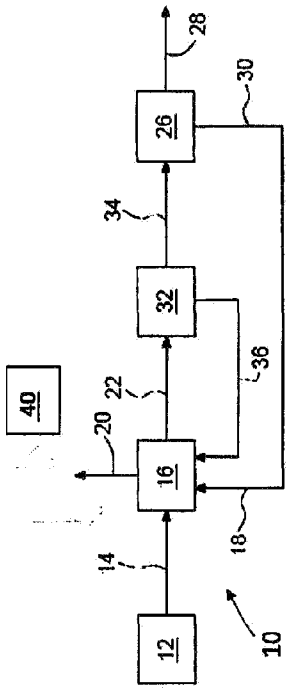

Figure 12

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  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)

```
  1 M G G G A H W G Y S G S I G P E H W G D
 21 L S P E Y L M C K I G K N Q S P I D I N
 41 S A D A V K A C L A P V S V Y Y V S D A
 61 K Y V V N N G H T I K V V M G G R G Y V
 81 V V D G K R F Y L K Q F H F H A P S E H
101 T V N G K H Y P F E A H F V H L D K N G
121 N I T V L G V F F K V G K E N P E L E K
141 V W R V M P E E P G Q K R H L T A R I D
161 P E K L L P E N R D Y Y R Y S G S L T T
181 P P C S E G V R W I V F K E P V E M S R
201 E Q L E K F R K V M G F D N N R P V Q P
221 L N A R K V M K *
```

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)

```
  1 M E H E - - W G Y S G S I G P E H W G D
 21 L S P E Y L M C K I G K N Q S P I D I N
 41 S A D A V K A C L A P V S V Y Y V S D A
 61 K Y V V N N G H T I K V V M G G R G Y V
 81 V V D G K R F Y L K Q F H F H A P S E H
101 T V N G K H Y P F E A H F V H L D K N G
121 N I T V L G V F F K V G K E N P E L E K
141 V W R V M P E E P G Q K R H L T A R I D
161 P E K L L P E N R D Y Y R Y S G S L T T
181 P P C S E G V R W I V F K E P V E M S R
201 E Q L E K F R K V M G F D N N R P V Q P
221 L N A R K V M K *
```

Figure 16
(SEQ ID NO 6)

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1	M	E	H	E	W	G	Y	S	G	S	I	G	P	E	H	W	G	D	L	S	20
21	P	E	Y	L	M	C	K	I	G	K	N	Q	S	P	I	D	I	N	S	A	40
41	D	A	V	K	A	C	L	A	P	V	S	V	Y	Y	V	S	D	A	K	Y	60
61	V	V	N	N	G	H	T	I	K	V	V	M	G	G	R	G	Y	V	V	V	80
81	D	G	K	R	F	Y	L	K	Q	F	H	F	H	A	P	S	E	H	T	V	100
101	N	G	K	H	Y	P	F	E	A	H	F	V	H	L	D	K	N	G	N	I	120
121	T	V	L	G	V	F	F	K	V	G	K	E	N	P	E	L	E	K	V	W	140
141	R	V	M	P	E	E	P	G	Q	K	R	H	L	T	A	R	I	D	P	E	160
161	K	L	L	P	E	N	R	D	Y	Y	R	Y	S	G	S	L	T	T	P	P	180
181	C	S	E	G	V	R	W	I	V	F	K	E	P	V	E	M	S	R	E	Q	200
201	L	E	K	F	R	K	V	M	G	F	D	N	N	R	P	V	Q	P	L	N	220
221	A	R	K	V	M	K	*														227

Figure 17

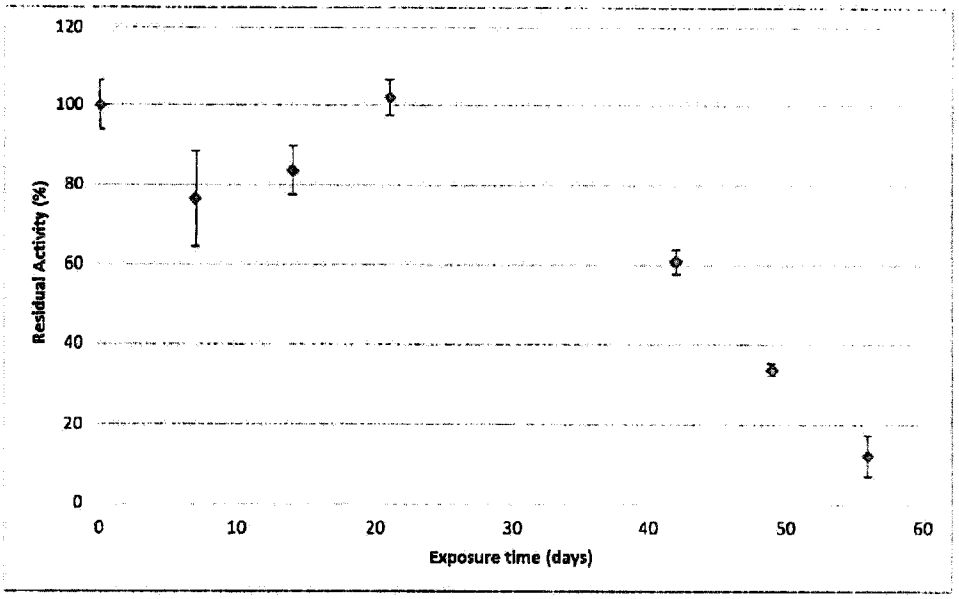

Figure 18

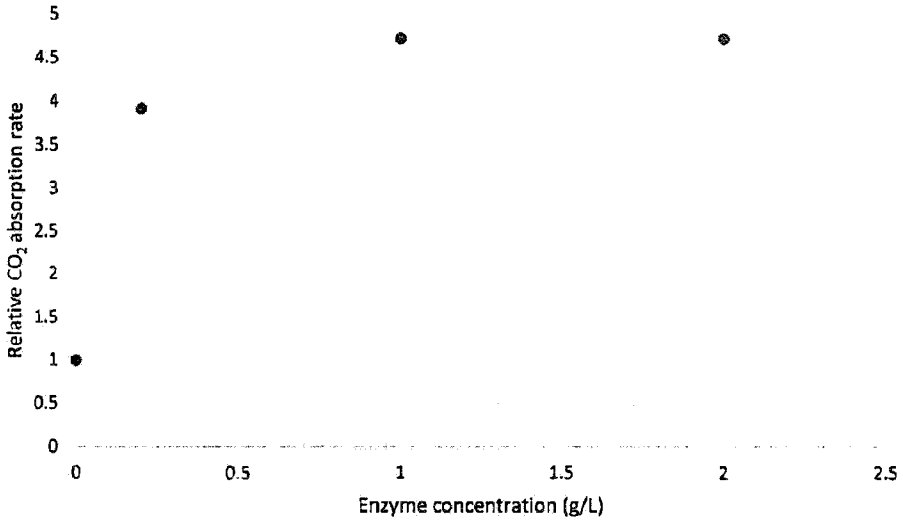

Figure 19

```
  1   M  E  H  E  W  S  Y  E  G  E  K  G  P  E  H  W  A  Q  L  K     20
  1   M  E  H  E  W  S  Y  E  G  E  K  G  P  E  H  W  A  F  L  R     20

 21   P  E  F  F  W  C  K  L  K  N  Q  S  P  I  N  I  D  K  K  Y     40
 21   P  E  F  F  W  C  K  L  K  N  Q  S  P  I  N  I  D  S  K  Y     40

 41   K  V  K  A  N  L  P  K  L  N  L  Y  Y  K  T  A  K  E  S  E     60
 41   K  V  K  A  N  L  P  K  L  N  L  Y  Y  K  T  A  L  E  S  E     60

 61   V  V  N  N  G  H  T  I  Q  I  N  I  K  E  D  N  T  L  N  Y     80
 61   V  V  N  N  G  H  T  I  Q  I  N  I  K  E  D  N  T  L  N  Y     80

 81   L  G  E  K  Y  Q  L  K  Q  F  H  F  H  T  P  S  E  H  T  I    100
 81   L  C  E  K  Y  Q  L  K  Q  F  H  F  H  T  P  S  E  H  T  V    100

101   E  K  K  S  Y  P  L  E  I  H  F  V  H  K  T  E  D  G  K  I    120
101   E  K  K  S  Y  P  L  E  I  H  F  V  H  K  T  E  D  G  K  I    120

121   L  V  V  G  V  M  A  K  L  G  K  T  N  K  E  L  D  K  I  L    140
121   L  V  V  G  V  M  A  K  L  G  K  T  N  K  E  L  D  K  I  L    140

141   N  V  A  P  A  E  E  G  E  K  I  L  D  K  N  L  N  L  N  N    160
141   N  V  A  P  A  E  E  G  E  K  I  L  D  K  N  L  N  L  N  N    160

161   L  I  P  K  D  K  R  Y  M  T  Y  S  G  S  L  T  T  P  P  C    180
161   L  I  P  K  D  K  R  Y  M  T  Y  S  G  S  L  T  T  P  P  C    180

181   T  E  G  V  R  W  I  V  L  K  K  P  I  S  I  S  K  Q  Q  L    200
181   T  E  G  V  R  W  I  V  L  K  K  P  I  S  I  S  K  Q  Q  L    200

201   E  K  L  K  S  V  M  V  N  P  N  N  R  P  V  Q  E  I  N  S    220
201   E  K  L  K  S  V  M  V  N  P  N  N  R  P  V  Q  E  I  N  S    220

221   R  W  I  I  E  G  F  *                                        228
221   R  W  I  I  E  G  F  *                                        228
```

Figure 20

top: SEQ ID NO 7 (SspCA)
bottom: SEQ ID NO 8 (6M1)