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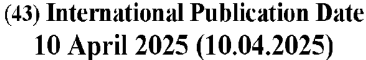

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(54) Title: SMALL MOLECULE ACTIVATORS

FIG. 1A

(57) Abstract: A catalyst composition comprising an oxidoreductase, a single electron oxidizer and a small molecule activator. A method comprising contacting a sugar with a catalyst composition comprising an oxidoreductase, a single electron oxidizer and a small molecule activator under conditions suitable for the formation of one or more oxidized sugar oxidation products.

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SMALL MOLECULE ACTIVATORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 63/588,577 filed October 6, 2023 entitled "SMALL MOLECULE ACTIVATORS," which is incorporated herein by reference in its entirety for all purposes.

REFERENCE TO SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML file format and is hereby incorporated by reference in its entirety. Said XML file, created on October 6,2023, is named "23ENZ003_3416-15400_ SEQYENCES.xml" and is 87,703 bytes in size.

TECHNICAL FIELD

[0003] The present disclosure generally relates to catalysts. More particularly, the present disclosure relates to small molecule activators for production of value-added chemicals.

BACKGROUND

[0004] Oxidoreductases are enzymes with a high potential for organic synthesis as their selectivity often exceeds the selectivity obtained when using comparable chemical catalysts (e.g., non-enzymatic catalysts). The biochemical cofactors utilized by oxidoreductases are typically inactivated and subsequently regenerated during the catalytic cycle. Cofactor regeneration is a point of vulnerability in the enzymatic cycle as it represents a point where the overall productivity of the enzyme is dependent on the extent to which a necessary cofactor can be recovered. The need for regeneration is considered a contributing factor in the low productivity levels observed for enzyme catalysts requiring cofactors.

[0005] Indirect electrochemical regeneration of enzymatic catalysts via a mediator may address the low productivity of electroenzymatic biotransformation processes as this approach offers an efficient and quasi mass-free method for providing the required redox equivalents.

[0006] For example, copper radical oxidases (CROs) are a class of non-flavoprotein alcohol oxidoreductases that employ molecular oxygen as a terminal electron acceptor to generate hydrogen peroxide. The CRO active site is typically composed of a single

copper, one tyrosine residue, and an unusual cross-linked cysteine−tyrosine unit that can be oxidized to form a stable tyrosyl-based protein radical. CROs become "inactivated" when the active copper center of the CRO is reduced from its catalytic copper(II) state to a copper(I) state, which eliminates the necessary radical species required for the enzyme's function, effectively shutting down its ability to oxidize substrates; this typically occurs through a single-electron reduction process and can be caused by factors like lack of oxygen or the presence of reducing agents. Occasionally, a single-electron reduction of the Cu(II)-coordinating tyrosyl radical occurs during catalysis, resulting in deactivation of the enzyme..

SUMMARY

[0007] Disclosed herein is a catalyst composition comprising an oxidoreductase, a single electron oxidizer and a small molecule activator.

[0008] Also disclosed herein is a method comprising contacting a sugar with a catalyst composition comprising an oxidoreductase, a single electron oxidizer and a small molecule activator under conditions suitable for the formation of one or more oxidized sugar oxidation products.

BRIEF DESCRIPTION OF THE FIGURES

[0009] The following figures form part of the present specification and is included to further demonstrate certain aspects of the present disclosure. The subject matter of the present disclosure may be better understood by reference to the figure in combination with the detailed description of specific aspects presented herein.

[0010] Figures 1A and 1B are schematic depictions of the active site mechanisms of galactose oxidase.

[0011] Figure 2 is a schematic depiction of the reaction of galactose oxidase catalyzed alcohol oxidation.

[0012] Figure 3 is a graph of the dissolved O_2 concentration as a function of the time and the concentration of 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS).

[0013] Figure 4 is a graph of the dissolved O_2 concentration as a function of time in a system comprising galactose oxidase, laccase, and ABTS.

[0014] Figures 5 is a relative activity comparison of horseradish peroxidase and DyP-type peroxidase as a function of pH.

[0015] Figure 6 is a comparison of conversions from reactions with horseradish peroxidase and dye-decolorizing peroxidase.

[0016] Figure 7 is a comparison of conversions from reactions with different isoforms of horseradish peroxidase.

[0017] Figure 8 is a comparison of conversion from reactions with different peroxidases.

[0018] Figure 9 is a comparison of glucose conversion from reactions with different concentrations of commercial horseradish peroxidases.

[0019] Figure 10 is a graph of the dissolved O_2 concentration as a function of the time and different peroxidases.

[0020] Figure 11 is a comparison of glucose conversion from reactions with different peroxidases.

[0021] Figure 12 is a comparison of glucose conversion from reactions with different small molecule activators.

DETAILED DESCRIPTION

[0022] Disclosed herein are small molecule activators (SMAs) that function to facilitate the complete transfer of two electrons to the active site of oxidoreductases such as CROs. In one or more aspects, the methods and compositions of the present disclosure result in the molecular transformation of feedstocks such as sugars, air, and carbon dioxide to value-added chemicals (VACs).

[0023] The processes of the present disclosure utilize a combination of biocatalysts and/or metal catalysts to perform one or more commercially important transformations such as oxidation, dehydration, carboxylation, carboligation, ammoxidation, and hydrogenation.

[0024] In an aspect of the present disclosure, a platform chemical is used to produce a VAC. In an aspect, the platform chemical comprises an alcohol or an aldehyde. Nonlimiting examples of platform chemicals include ethanol, methanol, a sugar such as glucose, hydroxymethylfurfural, 2,5-furandicarboxylic acid, glycerol, ethylene glycol, succinic acid, nicotinamide or combinations thereof.

[0025] In one or more aspects of the present disclosure, production of a VAC comprises enzymatic oxidation of a platform chemical such as a sugar. In such aspects, the enzymatic catalyst comprises an oxidoreductase such as a CRO.

[0026] Members of the CRO class include glyoxal oxidases (EC 1.1.3., GLOX) and galactose 6-oxidases (EC 1.1.3.9, GAO). GAO is a copper enzyme secreted by some fungal species, such as *Fusarium spp.*, to aid in the degradation of extracellular carbohydrate food sources through catalyzing the oxidation of primary alcohols to aldehydes while generating hydrogen peroxide. The native function of GAO is the

oxidation of D-galactose at the C6 position to generate D-galacto-hexodialdose. Although GAO is promiscuous, the native form is unable to bind glucose due to steric clashes with F464 and F194 in the active site and the equatorial C4 hydroxyl group on glucose.

[0027] Members of the GLOX family typically function on aldehydes such as methylglyoxal to produce acids. Members of the GAO class function on the C6 or similar alcohols of galactose or other sugars to produce aldehydes.

[0028] The GAO active site typically comprises a single copper atom coordinated to an axial tyrosine, two histidine residues, and an unusual cross-linked cysteine−tyrosine unit that can be oxidized to form a stable radical. Not intending to be bound by theory, a tryptophan stacked over the tyrosyl-cysteine is thought to account for further stabilization of the free radical. The catalytic cycle for GAO is split into two half-reactions. These reactions are depicted schematically in Figure 1 where HAT refers to hydrogen atom transfer; ET refers to electron transfer; and PT refers to proton transfer

[0029] With reference to Figure 1, in the first half reaction, a proton is transferred to the axial tyrosine anion, a hydrogen atom is transferred from the C6 of galactose to the tyrosyl-cysteine radical cofactor, and an electron is transferred from galactose to generate the aldehyde and Cu(I). In the first half of the reaction, the enzyme is reduced while oxidizing the alcohol to the aldehyde. In the second half reaction, an electron is transferred from Cu(I) to oxygen to form a superoxide, a hydrogen atom is transferred from the phenolic hydroxyl of the tyrosyl-cysteine cofactor to the superoxide to produce metal-bound hydroperoxide, and a proton is transferred from the axial tyrosine to hydroperoxide to produce hydrogen peroxide (O_2 to H_2O_2) and the reoxidized, active Cu(II) radical.

[0030] Occasionally, a single electron is transferred to the cysteine-tyrosine radical resulting in an inactive, “semi-reduced” GAO. This is depicted schematically in Figure 2. With reference to Figure 2, to rescue activity of the GAO, a peroxidase, such as the widely available biochemical research reagent, horseradish peroxidase (HRP), may be used to catalyze the 1e- transfer necessary to restore the tyrosyl-cysteine radical.

[0031] In one or more aspects, a catalytic composition comprising a CRO, a single electron oxidizer (SEO), and a SMA is used in the production of VACs. Without wishing to be limited by theory, the SEO performs a single electron oxidation on the SMA to generate the free radical form ($SMA^{\cdot+}$). The $SMA^{\cdot+}$ can reverse or return a CRO (e.g., GAO) to the active state. Without wishing to be limited by theory, reversing and/or

returning a CRO to the active state may comprise oxidation of one or more metals of the CRO at the active site of the enzyme to an active oxidation state.

[0032] In one or more aspects, an SMA suitable for use in this disclosure is a molecule that (i) is capable of stabilizing a free radical, (ii) can serve as a substrate for an SEO in a single electron oxidation reaction, and iii) is capable of restoring the active state of a CRO as evidenced by detectable activity (formation of product or oxygen consumption).

[0033] Nonlimiting examples of SMAs suitable for use in the present disclosure include L-tryptophan, 2-mercaptobenzothiazole, L-histidine, methylchloroisothiazolinone, o-dianisidine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 4-aminoantipyrine, L-tyrosine, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl, chloromethylisothiazolinone, 4-thiazolecarboxylic acid, Sunset yellow FCF, tartrazine, p-benzoquinone, dicoumarol, phthalimide, saccharin, phthalic anhydride, erythrosine B, 2-aminobenzothiazole, thiabendazole, 2-hydroxybenzothiazole, phenothiazine, 6-aminobenzothiazole, indigo carmine, naphthalimide, 2-aminothiazole, thiazole, 2H-1,4-benzothiazin-3(4H)-one, 2-oxindole, beta-lapachone, menaquinone, thiamine, 4-methyl-5-thiazoleethanol, Allura Red AC, menadione, p-cresol, Fast green FCF, Brilliant Blue FCF, methylisothiazolinone, caffeine, veratryl alcohol, fluorescein, and combinations thereof. The structures of several of these SMAs are depicted in Table 1.

Table 1

L-tryptophan	H N NH2 abs O OH
2-mercaptobenzothiazole	S S N H
L-histidine	H N O abs OH N NH2
Methylchloroisothiazolinone	Cl S N O

o-dianisidine	NH_2 O O H_2N
ABTS	NH_4^+ N N S O O O^- O S O^- O N S N N NH_4^+
4-aminoantipyrine	N N NH_2 O
L-tyrosine	O OH NH_2 HO
(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl	$O^\cdot$ N
chloromethylisothiazolinone	Cl S N O
4-thiazolecarboxylic acid	O N OH S
Sunset yellow FCF	O^- Na^+ O=S=O N N OH Na^+ O S O O^-
Tartrazine	Na^+ O^- S O O O N N N N O S O O O^- O Na^+ Na^+

p-benzoquinone	
dicoumarol	
phthalimide	
saccharin	
phthalic anhydride	
Erythrosine B	
2-aminobenzothiazole	
benzothiazole	
thiabendazole	
2-Hydroxybenzothiazole	
Phenothiazine	
6-aminobenzothiazole	

Indigo carmine	
naphthalimide	
2-aminothiazole	
thiazole	
2H-1,4-Benzothiazin-3(4H)-one	
2-oxindole	
beta-lapachone	
menaquinone	
thiamine	
4-Methyl-5-thiazoleethanol	
Allura Red AC	

Menadione	
p-cresol	
Fast green FCF	
Brilliant Blue FCF	
Methylisothiazolinone	
caffeine	
veratryl alcohol	
fluorescein	

[0034] A SEO suitable for use in the present disclosure can be an enzyme or chemical compound (e.g., peroxidase or ferricyanide, catalase, and the like). The SEO can be any molecule that can elicit the radical state of the SMA by performing a single electron oxidation. In one or more aspects, the SEO is an enzyme. For example, the SEO is an enzyme that can use H_2O_2 as the oxidant which is advantageous as H_2O_2 is produced as a coproduct by a CRO.

[0035] In one or more aspects, the SEO is an enzyme such as a laccase, horseradish peroxidase, Dyp-type peroxidase, lactoperoxidase, chloroperoxidase, manganese peroxidase 1, ascorbate peroxidase, dye-decolorizing peroxidase, unspecific peroxygenase, dehaloperoxidase, catalase-peroxidase, lignin peroxidase, soybean seed coat peroxidase, isoforms thereof or combinations thereof.

[0036] In one or more aspects, a method of the present disclosure a catalytic composition comprises an oxidoreductase, an SEO and a SMA. The catalytic composition may comprise an oxidoreductase in an amount ranging from about 0.01 g/L to about 1 g/L, additionally or alternatively, from about 0.1 g/L to about 1 g/L, additionally or alternatively, from about 0.2 g/L to about 1 g/L, additionally or alternatively, from about 0.4 g/L to about 1 g/L, additionally or alternatively, from about 0.6 g/L to about 1 g/L, additionally or alternatively, from about 0.75 g/L to 1 g/L, additionally or alternatively, about 0.01 g/L, about 0.05 g/L, about 0.1 g/L, about 0.2 g/L, about 0.3 g/L, about 0.4 g/L, about 0.5 g/L, about 0.6 g/L, about 0.7 g/L, about 0.8 g/L, about 0.9 g/L or, additionally or alternatively, about 1 g/L. The catalytic composition may comprise an SEO in an amount ranging from about 1 mg/L to about 250 mg/L, additionally or alternatively, from about 5 mg/L to about 250 mg/L, additionally or alternatively, from about 10 mg/L to about 250 mg/L, additionally or alternatively, from about 25 mg/L to about 250 mg/L, additionally or alternatively, from about 50 mg/L to find 250 mg/L, additionally or alternatively, from about 75 mg/L to 250 mg/L, additionally or alternatively, from about 100 mg/L to 250 mg/L, additionally or alternatively, from about 150 mg/L to 250 mg/L, additionally or alternatively, about 1 mg/L, about 5 mg/L, about 10 mg/L, about 15 mg/L, about 20 mg/L, about 25 mg/L, about 30 mg/L, about 35 mg/L, about 40 mg/L, about 45 mg/L, about 50 mg/L, about 55 mg/L, about 60 mg/L, about 65 mg/L, about 70 mg/L, about 75 mg/L, about 80 mg/L, about 85 mg/L, about 90 mg/L, about 95 mg/L, about 100 mg/L, about 105 mg/L, about 110 mg/L, about 115 mg/L, about 120 mg/L, about 125 mg/L, about 130 mg/L, about 135 mg/L, about 140 mg/L, about 145 mg/L, about 150 mg/L, about 155 mg/L, about 160 mg/L, about 165

mg/L, about 170 mg/L, about 175 mg/L, about 180 mg/L, about 185 mg/L, about 190 mg/L, about 195 mg/L, about 200 mg/L, about 205 mg/L, about 210 mg/L, about 215 mg/L, about 220 mg/L, about 225 mg/L, about 230 mg/L, about 235 mg/L, about 240 mg/L, about 245 mg/L or, additionally or alternatively, about 250 mg/L. The catalytic composition may comprise an SMA in an amount ranging from about 1 ppm to about 500 ppm, additionally or alternatively, from about 5 ppm to about 500 ppm, additionally or alternatively, from about 10 ppm to about 500 ppm, additionally or alternatively, from about 20 ppm to about 500 ppm, additionally or alternatively, or from about 40 ppm to about 400 ppm, additionally or alternatively, from about 50 ppm to about 350 ppm additionally or alternatively, from about 75 ppm to about 200 ppm additionally or alternatively, about 1 ppm, about 5 ppm, about 10 ppm, about 15 ppm, about 20 ppm, about 25 ppm, about 30 ppm, about 35 ppm, about 40 ppm, about 45 ppm, about 50 ppm, about 55 ppm, about 60 ppm, about 65 ppm, about 70 ppm, about 75 ppm, about 80 ppm, about 85 ppm, about 90 ppm, about 95 ppm, about 100 ppm, about 105 ppm, about 110 ppm, about 115 ppm, about 120 ppm, about 125 ppm, about 130 ppm, about 135 ppm, about 140 ppm, about 145 ppm, about 150 ppm, about 155 ppm, about 160 ppm, about 165 ppm, about 170 ppm, about 175 ppm, about 180 ppm, about 185 ppm, about 190 ppm, about 195 ppm, about 200 ppm, about 205 ppm, about 210 ppm, about 215 ppm, about 220 ppm, about 225 ppm, about 230 ppm, about 235 ppm, about 240 ppm, about 245 ppm, about 250 ppm, about 255 ppm, about 260 ppm, about 265 ppm, about 270 ppm, about 275 ppm, about 280 ppm, about 285 ppm, about 290 ppm, about 295 ppm, about 300 ppm, about 305 ppm, about 310 ppm, about 315 ppm, about 320 ppm, about 325 ppm, about 330 ppm, about 335 ppm, about 340 ppm, about 345 ppm, about 350 ppm, about 355 ppm, about 360 ppm, about 365 ppm, about 370 ppm, about 375 ppm, about 380 ppm, about 385 ppm, about 390 ppm, about 395 ppm, about 400 ppm, about 405 ppm, about 410 ppm, about 415 ppm, about 420 ppm, about 425 ppm, about 430 ppm, about 435 ppm, about 440 ppm, about 445 ppm, about 450 ppm, about 455 ppm, about 460 ppm, about 465 ppm, about 470 ppm, about 475 ppm, about 480 ppm, about 485 ppm, about 490 ppm, about 495 ppm, or, additionally or alternatively, about 500 ppm.

[0037] Disclosed herein are methods and compositions in which the addition of a small molecule electron shuttle dramatically increases the velocity of the reaction and can lead to higher yields in enzyme cascades containing a CRO and a peroxidase or other single electron transferring enzymes. The present methods and compositions

beneficially lower product costs due to increased efficiency of enzyme regeneration and have lower mass requirements for a CRO, peroxidase, catalase, and other enzymes, etc. Currently production costs for CROs *in vitro* systems are high and are limited to applications such as generating fine chemicals (e.g., pharmaceuticals).

[0038] The present methods and compositions beneficially lower product costs due to increased efficiency of enzyme regeneration, and have lower mass requirements for a CRO, peroxidase, catalase, and other enzymes, etc. The presently disclosed SMA-mediated CRO enzyme regeneration increases the enzyme catalytic efficiency, leading to a higher reaction rate and productivity resulting in a decreased amount of enzyme used to achieve a user and/process desired conversion.

[0039] The presently disclosed catalytic compositions enable the industrial use of CROs (e.g., galactose oxidase) to produce specialty and commodity chemicals. Further, the present disclosure may result in increased efficiency of the enzymatic system for oxidation or other chemical transformations. For example, a catalyst composition of the present disclosure can be applied to other CRO catalyzed reactions in which the SMA mediated enzyme regeneration system will improve the CRO catalytic efficiency and improve the production rate. In an aspect, the oxidoreductase is GAO and the reaction is the production of glyoxal from ethylene glycol.

[0040] Herein, reference has been made to “sources” of enzyme. It is to be understood this refers to the biomolecule as expressed by the named organism. It is contemplated the enzyme may be obtained from the organism or a version of said enzyme (wildtype or recombinant) and provided as a suitable construct to an appropriate expression system.

[0041] In an aspect, an enzyme of the type disclosed herein may be cloned into an appropriate expression vector and used to transform cells of an expression system such as *E. coli*, *Saccharomyces* sp., *Pichia sp.*, *Aspergillus* sp., *Trichoderma* sp., or *Myceliophthora* sp. A "vector" is a replicon, such as plasmid, phage, viral construct or cosmid, to which another DNA segment may be attached. Vectors are used to transduce and express a DNA segment in cells. As used herein, the terms "vector" and "construct" may include replicons such as plasmids, phage, viral constructs, cosmids, Bacterial Artificial Chromosomes (BACs), Yeast Artificial Chromosomes (YACs) Human Artificial Chromosomes (HACs) and the like into which one or more gene expression cassettes may be or are ligated. Herein, a cell has been "transformed" by an exogenous or heterologous nucleic acid or vector when such nucleic acid has been introduced inside

the cell, for example, as a complex with transfection reagents or packaged in viral particles. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell.

[0042] In an aspect, the gene of an enzyme disclosed herein is provided as a recombinant sequence in a vector where the sequence is operatively linked to one or more control or regulatory sequences. "Operatively linked" expression control sequences refer to a linkage in which the expression control sequence is contiguous or substantially contiguous with the gene of interest so as to control the gene of interest, as well as expression control sequences that act in trans or at a distance to control the gene of interest.

[0043] The terms "expression control sequence" and/or "regulatory sequences" are used interchangeably and are used herein to refer to polynucleotide sequences which affect the expression of coding sequences to which they are operatively linked. Expression control sequences are sequences that control the transcription, post-transcriptional events, and translation of nucleic acid sequences. Expression control sequences include appropriate transcription initiation, termination, promoter, and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (e.g., ribosome binding sites); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence. The term "control sequence" is intended to include, at a minimum, a component whose presence is essential for expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0044] The term "recombinant host cell" ("expression host cell", "expression host system", "expression system" or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term

"host cell" as used herein. A recombinant host cell may be an isolated cell or cell line grown in culture or may be a cell which resides in a living tissue or organism.

[0045] In one or more aspects, a CRO suitable for use in the present disclosure is a CRO defined by of any of SEQ ID Nos. 1 through 39. In one or more aspects, a CRO suitable for use in the present disclosure has from about 85% to about 100% sequence identity with any of SEQ ID Nos. 1 through 39.

[0046] In one or more aspects, a catalase suitable for use in the present disclosure is a catalase defined by of any of SEQ ID Nos. 40 through 44. In one or more aspects, a peroxidase suitable for use in the present disclosure is a peroxidase defined by of any of SEQ ID Nos. 45 through 62

[0047] In an aspect, method of the present disclosure comprises contacting a catalyst composition of the type disclosed herein (e.g., GAO, SMA, SEO) with a sugar under conditions suitable for the formation of one or more sugar oxidation products. In some aspects, the sugar comprises glucose and the one or more sugar oxidation products (SOPs) comprise aldonic acid, uronic acid, aldaric acid, or combinations thereof, and a counter cation.

[0048] Additionally or alternatively, in an aspect, the one or more sugar oxidation products comprise a glucose oxidation product, a gluconic acid oxidation product, a gluconate, or combinations thereof. The glucose oxidation product, gluconic acid oxidation product, or combinations thereof, may be buffered to a suitable pH.

[0049] Additionally or alternatively, the one or more sugar oxidation products comprise glucaric acid, gluconic acid, glucuronic acid, glucose oxidation products, gluconic acid oxidation products, or combinations thereof. Additionally or alternatively, in one or more aspects, the one or more sugar oxidation product comprises disaccharides, oxidized disaccharides, uronic acid, aldaric acid, or combinations thereof.

[0050] Additionally or alternatively, the one or more sugar oxidation products comprise gluconic acid, glucaric acid, glucuronic acid, n-keto-acids, C2 to C6 diacids, or combinations thereof.

[0051] Additionally or alternatively, the one or more sugar oxidation products comprise galactonic acid, galactaric acid, an oxidation product comprising predominantly (e.g., greater than about 50 weight percent) galactonic acid and/or galactaric acid with minor component species of n-keto-acids, C2 to C6 diacids, or combinations thereof. Additionally or alternatively, in one or more aspects, the one or more sugar oxidation

products comprise glutamic acid. Additionally or alternatively, the one or more sugar oxidation products comprise glucodialdose, 2-ketoglucose, or combinations thereof.

[0052] In such aspects, the buffered glucose oxidation product, the buffered gluconic acid oxidation product, or combinations thereof are buffered to a suitable pH. For example, the glucose oxidation product, gluconic acid oxidation product or combination thereof may be buffered to a pH in the range of from about 1 to about 5. Buffering of the one or more sugar oxidation product may be carried using any suitable acid, base, or combination thereof.

[0053] In one or more aspects, the one or more sugar oxidation products comprise aldonic acid, uronic acid, aldaric acid, a gluconic acid oxidation product, a gluconate, glucaric acid, gluconic acid, glucuronic acid, glucose oxidation products, galactonic acid, galactaric acid, glutamic acid, a lactone of gluconic acid, a lactone of glucaric acid, a lactone of galactaric acid, a lactone of galactonic acid, glucodialdose, 2-ketoglucose, disaccharides, oxidized disaccharides, n-keto-acids, C2 to C6 diacids, salts thereof or combinations thereof.

[0054] In an aspect, the one or more sugar oxidation products comprise a glucose oxidation product, a gluconic acid oxidation product, a gluconate, glucaric acid, an oxidized glucuronolactone, a uronic acid oxidation product, or combinations thereof. Additionally or alternatively, the one or more sugar oxidation product comprises a buffered glucose oxidation product, a buffered gluconic acid oxidation product or combinations thereof. In some such aspects, the buffered glucose oxidation product, the buffered gluconic acid oxidation product, or combinations thereof are buffered to a pH within a range disclosed herein with any suitable acid or base such as sodium hydroxide. In an example of such aspects, the one or more sugar oxidation product comprises a mixture of gluconic acid and glucaric acid and further comprises a minor component species comprising n-keto-acids, C2-C6 diacids or combinations thereof.

[0055] In an aspect, the one or more sugar oxidation products comprise glucodiamine, glucodialdose, 2-ketoglucose, glucaric acid, lactones of glucaric acid, gluconic acid, lactones of gluconic acid, galactonic acid, lactones of galactonic acid, galactaric acid, lactones of galactaric acid, galactonic acid glucoheptonic acid, lactones of glucoheptonic acid, or combinations thereof.

[0056] SOPs disclosed herein may function as or be useful in the production of VAC.

ADDITIONAL DISCLOSURE

[0057] A first aspect which is a catalyst composition comprising an oxidoreductase, a single electron oxidizer and a small molecule activator.

[0058] A second aspect which is the composition of the first aspect wherein the oxidoreductase comprises a copper radical oxidase.

[0059] A third aspect which is the composition of any of the first through second aspects wherein the oxidoreductase is selected from the group consisting essentially of a galactose oxidase, a glyoxal oxidase, an alcohol oxidase, mutants thereof and combinations thereof.

[0060] A fourth aspect which is the composition of any of the first through third aspects wherein the oxidoreductase has any of SEQ ID No. 1 through SEQ ID No. 39.

[0061] A fifth aspect which is the composition of any of the first through fourth aspects wherein the oxidoreductase is present in an amount ranging from about 0.01 g/L to about 1 g/L.

[0062] A sixth aspect which is the composition of any of the first through fifth aspects wherein the small molecule activator comprises include L-tryptophan, 2-mercaptobenzothiazole, L-histidine, methylchloroisothiazolinone, o-dianisidine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 4-aminoantipyrine, L-tyrosine, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl, chloromethylisothiazolinone, 4-thiazolecarboxylic acid, Sunset yellow FCF, tartrazine, p-benzoquinone, dicoumarol, phthalimide, saccharin, phthalic anhydride, erythrosine B, 2-aminobenzothiazole, thiabendazole, 2-hydroxybenzothiazole, phenothiazine, 6-aminobenzothiazole, indigo carmine, naphthalimide, 2-aminothiazole, thiazole, 2H-1,4-benzothiazin-3(4H)-one, 2-oxindole, beta-lapachone, menaquinone, thiamine, 4-methyl-5-thiazoleethanol, Allura Red AC, menadione, p-cresol, Fast green FCF, Brilliant Blue FCF, methylisothiazolinone, caffeine, veratryl alcohol, fluorescein, or combinations thereof.

[0063] A seventh aspect which is the composition of any of the first through fifth aspects wherein the small molecule activator is present in an amount ranging from about 1 ppm to about 500 ppm.

[0064] An eighth aspect which is the composition of any of the first through seventh aspects wherein the single electron oxidizer comprises laccase, horseradish peroxidase, dyp-type peroxidase, lactoperoxidase, chloroperoxidase, manganese peroxidase 1, ascorbate peroxidase, dye-decolorizing peroxidase, unspecific

peroxygenase, dehaloperoxidase, catalase-peroxidase, lignin peroxidase, soybean seed coat peroxidase, isoforms thereof or combinations thereof.

[0065] A ninth aspect which is the composition of any of the first through eighth aspects wherein the single electron oxidizer has any SEQ ID No.45 through SEQ ID No. 62.

[0066] A tenth aspect which is the composition of any of the first through ninth aspects wherein the single electron oxidizer is present in an amount ranging from about 1 mg/L to about 250 mg/L.

[0067] An eleventh aspect which is the composition of any of the first through tenth aspects further comprising a catalase.

[0068] A twelfth aspect which is the composition of the eleventh aspect wherein the catalase has any SEQ ID No. 40 through SEQ ID No. 44.

[0069] A thirteenth aspect which is a method comprising contacting a sugar with a catalyst composition comprising an oxidoreductase, a single electron oxidizer and a small molecule activator under conditions suitable for the formation of one or more oxidized sugar oxidation products.

[0070] A fourteenth aspect which is a method of the thirteenth aspect wherein the oxidoreductase is selected from the group consisting essentially of a galactose oxidase, a glyoxal oxidase, an alcohol oxidase, mutants thereof and combinations thereof.

[0071] A fifteenth aspect which is the method of any of the thirteenth through fourteenth aspects wherein the oxidoreductase has any of SEQ ID No. 1 through SEQ ID No. 39.

[0072] A sixteenth aspect which is the method of any of the thirteenth through fifteenth aspects wherein the small molecule activator comprises include L-tryptophan, 2-mercaptobenzothiazole, L-histidine, methylchloroisothiazolinone, o-dianisidine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 4-aminoantipyrine, L-tyrosine, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl, chloromethylisothiazolinone, 4-thiazolecarboxylic acid, Sunset yellow FCF, tartrazine, p-benzoquinone, dicoumarol, phthalimide, saccharin, phthalic anhydride, erythrosine B, 2-aminobenzothiazole, thiabendazole, 2-hydroxybenzothiazole, phenothiazine, 6-aminobenzothiazole, indigo carmine, naphthalimide, 2-aminothiazole, thiazole, 2H-1,4-benzothiazin-3(4H)-one, 2-oxindole, beta-lapachone, menaquinone, thiamine, 4-methyl-5-thiazoleethanol, Allura Red AC, menadione, p-cresol, Fast green FCF, Brilliant Blue FCF, methylisothiazolinone, caffeine, veratryl alcohol, fluorescein, or combinations thereof.

[0073] A seventeenth aspect which is the method of any of the thirteenth through sixteenth aspects wherein the single electron oxidizer comprises laccase, horseradish

peroxidase, dyp-type peroxidase, lactoperoxidase, chloroperoxidase, manganese peroxidase 1, ascorbate peroxidase, dye-decolorizing peroxidase, unspecific peroxygenase, dehaloperoxidase, catalase-peroxidase, lignin peroxidase, soybean seed coat peroxidase, isoforms thereof or combinations thereof.

[0074] An eighteenth aspect which is the method of any of the thirteenth through seventeenth aspects wherein the single electron oxidizer has any SEQ ID No.45 through SEQ ID No. 62.

[0075] A nineteenth aspect which is the method of any of the thirteenth through eighteenth aspects wherein the sugar comprises glucose.

[0076] A twentieth aspect which is the method of any of the thirteenth through nineteenth aspects wherein the one or more sugar oxidation products comprise aldonic acid, uronic acid, aldaric acid, a gluconic acid oxidation product, a gluconate, glucaric acid, gluconic acid, glucuronic acid, glucose oxidation products, galactonic acid, galactaric acid, glutamic acid, a lactone of gluconic acid, a lactone of glucaric acid, a lactone of galactaric acid, a lactone of galactonic acid, glucodialdose, 2-ketoglucose, disaccharides, oxidized disaccharides, n-keto-acids, C2 to C6 diacids, salts thereof or combinations thereof.

EXAMPLES

[0077] The aspects having been generally described, the following example is given as particular aspects of the disclosure and to demonstrate the practice and advantages thereof. It is understood that the example is given by way of illustration and is not intended to limit the specification or the claims in any manner.

EXAMPLE 1

HRP-ABTS activation of a GAO mutant

[0078] ABTS was investigated as an electron mediator for GAO regeneration. The activity of GAO variants on glucose oxidation was tested by O_2 consumption assay, and a dissolved O_2 monitoring device, RESIPHER, was used to measure O_2 consumption in the assay. RESIPHER is a handheld cell culture monitor that measures oxygen consumption in standard multi-well plates commercially available from Lucid Scientific. The O_2 consumption assay was set up with 50 mM Na phosphate, at a pH of 8, 20 g/L glucose, 15 mg/L catalase, 50 µM of $CuSO_4$, 0.4 mg/L of GAO variant, and different concentrations of ABTS ranging from 0-50 ppm. These results are presented in Figure 3.

[0079] With reference to Figure 3, without the activator molecule no O_2 consumption was detected. The addition of ABTS in the assay clearly activates GAO and initiates the reaction. Notably, the activity of GAO increases with increasing ABTS concentration.

EXAMPLE 2

Laccase-ABTS activation of GAO

[0080] Laccase-ABTS was investigated as an electron mediator for GAO regeneration. Laccase is a multi-copper oxidase that catalyzes the oxidation of one electron of a wide range of aromatic compounds, including ABTS. Laccase was tested for GAO activation through oxidizing ABTS and generating an ABTS radical. The O_2 consumption assay was set up with 50 mM Na phosphate, pH of 8, 20 g/L glucose, 15 mg/L catalase, 50 μM of $CuSO_4$, with or without 4 mg/L of GAO variant, with or without 10 ppm of ABTS, and with or without 100 mg/L of laccase from *Trametes versicolor* (purchased from Sigma). Specifically, Sample 1 contained GAO, laccase and ABTS; Sample 2 contained GAO and laccase (No ABTS); Sample 3 contained GAO and ABTS (No laccase); and Sample 4 contained laccase and ABTS (No GAO). The results are presented in Figure 4. The O_2 consumption was clearly detected from the assay with GAO, ABTS, and laccase, and no O_2 consumption was detected in the assays are missing any of three components.

EXAMPLE 3

[0081] DyP-type peroxidase from *streptomyces lividans*-SMA activation of GAO

[0082] Activation of GAO by DyP-type peroxidase from *Streptomyces lividans* was investigated. DtpA has been previously identified to activate GlxA, a copper-dependent, primary alcohol oxidase from *Streptomyces lividans*. GlxA's active site consists of a mononuclear Cu ion and a cross-linked cysteine−tyrosine unit and is active at pH4.0. DtpA was tested for GAO activation in the reactor. The reaction was set up with 50 mM Na phosphate, pH 8.0, 15 wt.% glucose, 0.01 g/L GAO, 0.05 g/L catalase, 0.5 g/L or 1 g/L DtpA, 6.6 μM or 1 ppm SMA, and 50 μM $MnSO_4$. The positive control reaction was set up similarly, but with 0.005 g/L HRP, instead of DtpA. The reactor was pressured with pure O_2 at 100 psi and stirred for 24 hrs.

[0083] The results are presented in Figures 5 and 6. With reference to Figures 5 and 6, the percentage conversion of glucose to glucodialdose was calculated based on the weight percent (wt.%) of glucose after 0 and 24 hours. At 1 g/L, DtpA was able to activate GAO, with 25% conversion of glucose to GDA after 24 hours of reaction.

However, it was not as efficient as HRP since HRP can activate GAO at a much lower concentration with a much higher conversion percentage.

EXAMPLE 4

[0084] Activation of GAO by multiple isoforms of Horseradish peroxidase from *Amoracia rusticana*

[0085] The ability of various isoforms of horseradish peroxidase from *Amoracia rusticana* to activate GAO was investigated. Horseradish (*Amoracia rusticana*) expresses multiple isoforms of heme peroxidases, including the most common and well characterized, C1A. C1A. The isoforms C2 and E5 were tested in a reactor for conversion activity under the following conditions: 50mM sodium phosphate, pH 8.0, 15% dextrose (w/v), 0.01 g/L GAO, 0.1 g/L catalase, 0.0025 g/L HRP-C2 or HRP-E5, 0.01mM $CuSO_4$, 6.6 µM or 1 ppm SMA, and 50 µM $MnSO_4$. Reactors were run for 72 hours after which the concentration of dextrose and glucodialdose were measured. These concentrations were then used to calculate total conversion. A positive control with HRP-C1A and a negative control with no HRP were run under the same conditions. HRP-C2 demonstrated equivalent capacity to convert dextrose as compared to the control, C1A. These results are presented in Figure 7.

EXAMPLE 5

[0086] Activation of GAO with SMA coupled with other peroxidases.

[0087] The reaction is set up with 50 mM Na phosphate, pH 8.0, 15 wt.% glucose, 0.02 g/L GAO variant, 0.05 g/L catalase, 6.6 µM or 1 ppm SMA, 50 µM $MnSO_4$, and 0.005 g/L of different type of peroxidases, including lactoperoxidase (LPO), chloroperoxidase (CholP), and manganese peroxidase (MnP). The positive control reaction is set up similarly, but with 0.005 g/L HRP. The reactor is pressured with pure O_2 at 100 psi and stirred for 24 hrs. The conversion % of glucose to GDA is calculated based on the wt.% of glucose at time 0 and 24 hours. Compared to the negative control reaction without peroxidase, adding any of the peroxidases clearly activate GAO in the reaction, although its efficiency is not as good as HRP. These results are depicted in Figure 8.

EXAMPLE 6

[0088] Glucose conversion function of GAO enzyme activated by different concentrations of commercial HRP (HRP_GoldBio) and HRP C1A purified in house from the secretome of recombinant fungal culture.

[0089] Parr reactions were used to compare the function of HRP_GoldBio and in house expressed HRP-C1Ain activating GAO enzyme to convert glucose to GDA. The reaction

was performed at 11°C in O_2 pressurized (150 psi) Parr bomb reactors in 50 mM Na phosphate, pH 8.0, containing 10 mg/L GAO enzyme, 15% (w/v) D-glucose, 100 mg/L Katalase AK, 0.05 mM $MnSO_4$, and various concentrations (0.25 – 2 mg/L) of HRP_GoldBio and in house expressed HRP-C1A. Samples were collected at the beginning and 46 hours. Glucose and GDA were quantified by mass spectrometry. The glucose conversion rate was calculated by comparing the glucose concentration at the beginning and the end of the reaction. It was found that in house expressed HRP-C1A displayed a very comparable function as HRP_GoldBio in activating GAO enzyme in the Parr reactions based on their corresponding glucose conversion rate. These results are depicted in Figure 9.

EXAMPLE 7

[0090] Different peroxidases including HRP_GoldBio, IrLa DyP and PlSa DyP were investigated for their function in activating GAO enzyme in an oxygen consumption based assay. The experiment was set up in 50 mM Na phosphate, pH 8.0, containing 1 mg/L GAO enzyme, 15 mg/L Katalase AK, 1% (w/v) D-glucose, 10 mg/L HRP_GoldBio, IrLa DyP or PlSa DyP. The glucose oxidizing activity of GAO enzyme was measured by monitoring O_2 consumption with Resipher. It was found that IrLa DyP activated GAO enzyme as efficiently as HRP_GoldBio. In addition, PlSa DyP also significantly activates GAO enzyme though with lower potency than HRP_GoldBio and IrLa DyP. These results are depicted in Figure 10.

EXAMPLE 8

[0091] Glucose conversion function of GAO enzyme activated by different concentrations of commercial HRP (HRP_GoldBio), *Irpex lacteus* DyP (IrLa DyP) or *Pleurotus sapidus* DyP (PlSa DyP)

[0092] Parr reactions were used to compare the function of HRP_GoldBio, IrLa DyP or PlSa DyP in activating GAO enzyme to convert glucose to GDA. The reactions were performed at 11 °C in O_2 pressurized (150 psi) Parr bomb reactors in 50 mM Na phosphate, pH 8.0, containing 10 mg/L GAO enzyme, 15% (w/v) D-glucose, 100 mg/L Katalase AK, 0.05 mM $MnSO_4$, and one of two concentrations of (1.25 mg/L and 2.5 mg/L) of HRP_GoldBio or IrLa DyP or 5 mg/L PlSa DyP. Specifically, Sample 5 contained 1.25 mg/L of HRP_GoldBio; Sample 6 contained 2.5 mg/L of HRP_GoldBio; Sample 7 contained 1.25 mg/L of IrLa DyP; Sample 8 contained 2.5 mg/L of IrLa DyP; and Sample 9 contained 5 mg/L of PlSa DyP. Samples were collected at the beginning,

24 hours and 140 hours of the reaction. Glucose and GDA were quantified by mass spectrometry. The glucose conversion rate was calculated by comparing the glucose concentration at the beginning and different times of the reaction. It was found that all of three tested peroxidases were able to activate GAO enzyme in Parr bomb reactions although with differential potency. In addition, although GAO converted less glucose within 24 hours with either DyP enzyme than with HRP_GoldBio, it converted similar level of glucose at 140 hours in reactions with IrLa DyP and HRP_GoldBio, especially when a 2.5 mg/L concentration of peroxidase was used. These results are depicted in Figure 11.

EXAMPLE 9

[0093] Activation of GAO with HRP coupled with other SMAs

[0094] The reaction is set up with 50 mM Na phosphate, pH 8.0, 30 wt.% glucose, 0.02 g/L GAO variant, 0.05 g/L catalase, different SMAs (including , fluorescein, Rhodamine B, Erythrosine B, tyrosine, and phenol), 50 µM $MnSO_4$, and 0.005 g/L of HRP. The control reaction is set up similarly, but without any activator molecule. The reactor is pressured with air at 135 psig and stirred for 24 hrs. The conversion % of glucose to GDA is calculated based on the wt.% of glucose at time 0 and 24 hours. Compared to the negative control reaction without SMA, the reaction conversion is boosted by adding the SMAs (including Erythrosine B, fluorescein, Rhodamine B, and tyrosine). Interestingly, adding phenol to the reaction negatively affects the reaction performance. These results are depicted in Figure 12.

EXAMPLE 10

[0095] Screening of small molecule GAO activators

[0096] In order to rapidly assess GAO activation abilities of candidate small molecules, oxygen consumption assays were performed using a Lucid Scientific RESIPHER, a microplate-compatible O_2 sensing platform. Reactions were set up with a total volume of 300 uL, with 50 mM Na phosphate, pH 8.0, 50 uM $CuSO_4$, 15 mg/L buffer-exchanged Katalase-AK, 5 mg/L HRP, 2 wt.% glucose, 0.5 mg/L GAO, and a variable concentration of small molecule activator candidate (generally between 1-50 ppm) in technical duplicate. Oxygen concentrations were monitored every 1 minute for 1-2 hr. Oxygen concentration data was downloaded from the Lucid Scientific cloud server, and all data from the first 4 minutes of the assay were omitted from analysis to exclude effects from mixing of assay stock solutions. For each experiment, linear regressions were performed in six-point moving windows across the 4-minute through 25-minute

timepoints of the assay. Fitted curves with R^2 values less than 0.96 were discarded, and among curves that exceeded this R^2 threshold, the most-negative curve for each experiment (i.e., with the highest computed oxygen consumption rate) was used as the oxygen consumption rate (OCR) value for the experiment. For experiments that had positive computed OCRs, these values were set to zero. These results are depicted in Table 2.

Table 2

Compound group	Compound	CAS	Concentration (ppm)	Oxygen consumption rate (uM/min)	OCR %CV
None	No activator		0	0.00	0.0%
aniline	o-dianisidine	119-90-4	1	0.00	0.0%
aniline	o-dianisidine	119-90-4	50	0.00	0.0%
anisole	veratryl alcohol	93-03-8	1	0.00	0.0%
anisole	veratryl alcohol	93-03-8	50	0.00	0.0%
azo dye	ABTS	30931-67-0	5	-3.39	3.0%
azo dye	ABTS	30931-67-0	50	-3.56	1.1%
azo dye	Allura Red AC	25956-17-6	5	0.00	0.0%
azo dye	Allura Red AC	25956-17-6	50	0.00	0.0%
azo dye	Sunset Yellow FCF	2783-94-0	5	0.00	0.0%
azo dye	Sunset Yellow FCF	2783-94-0	50	0.00	0.0%
azo dye	Tartrazine	1934-21-0	5	0.00	0.0%
azo dye	Tartrazine	1934-21-0	50	0.00	0.0%
indigo dye	Indigo carmine	860-22-0	5	0.00	0.0%
indigo dye	Indigo carmine	860-22-0	50	0.00	0.0%
isothiazole	ProClin 150		5	0.00	0.0%
isothiazole	ProClin 150		50	0.00	0.0%
isothiazole	ProClin 200		5	0.00	0.0%
isothiazole	ProClin 200		50	0.00	0.0%
isothiazole	ProClin 300		5	0.00	0.0%
isothiazole	ProClin 300		50	0.00	0.0%
isothiazole	ProClin 950		5	0.00	0.0%
isothiazole	ProClin 950		50	-0.27	43.5%
misc. N-heterocycle	3-amino-1,2,4-triazole	61-82-5	1	0.00	0.0%
misc. N-heterocycle	3-amino-1,2,4-triazole	61-82-5	50	0.00	0.0%
misc. N-heterocycle	4-aminoantipyrine	83-07-8	1	0.00	0.0%

misc. N-heterocycle	4-aminoantipyrine	83-07-8	50	0.00	0.0%
misc. N-heterocycle	L-tryptophan	73-22-3	1	0.00	0.0%
misc. N-heterocycle	L-tryptophan	73-22-3	50	0.00	0.0%
misc. N-heterocycle	caffeine	58-08-2	1	0.00	0.0%
misc. N-heterocycle	caffeine	58-08-2	50	-0.10	19.5%
misc. N-heterocycle	phenothiazine	92-84-2	5	-0.13	15.3%
misc. N-heterocycle	phenothiazine	92-84-2	50	-0.49	0.4%
misc. N-heterocycle	phthalimide	85-41-6	5	0.00	0.0%
misc. N-heterocycle	phthalimide	85-41-6	50	0.00	0.0%
phenol	1, 4-benzoquinone	106-51-4	5	0.00	0.0%
phenol	1, 4-benzoquinone	106-51-4	50	-0.08	4.9%
phenol	L-tyrosine	60-18-4	1	-0.52	8.2%
phenol	L-tyrosine	60-18-4	50	-1.11	12.8%
phenol	eugenol	97-53-0	1	0.00	0.0%
phenol	eugenol	97-53-0	50	0.00	0.0%
phenol	o-cresol	95-48-7	1	-0.11	3.4%
phenol	o-cresol	95-48-7	50	-0.22	0.6%
phenol	p-cresol	106-44-5	5	-0.40	0.0%
phenol	p-cresol	106-44-5	50	-0.50	5.5%
phenol	phenol	108-95-2	1	0.00	0.0%
phenol	phenol	108-95-2	50	-0.74	1.6%
phenol	pyrogallol	87-66-1	1	0.00	0.0%
phenol	pyrogallol	87-66-1	50	-1.46	6.4%
thiazole	1,3-benzothiazole 6-amine	533-30-2	5	-0.58	7.6%
thiazole	1,3-benzothiazole 6-amine	533-30-2	50	-0.21	9.4%
thiazole	2-aminobenzothiazole	136-95-8	1	0.00	0.0%
thiazole	2-aminobenzothiazole	136-95-8	50	0.00	0.0%
thiazole	2-aminothiazole	96-50-4	1	0.00	0.0%
thiazole	2-aminothiazole	96-50-4	50	-0.60	4.2%

thiazole	2-hydroxybenzothiazole	934-34-9	1	0.00	0.0%
thiazole	2-hydroxybenzothiazole	934-34-9	50	-0.36	2.5%
thiazole	4-methyl-5-thiazoleethanol	137-00-8	5	0.00	0.0%
thiazole	4-methyl-5-thiazoleethanol	137-00-8	50	0.00	0.0%
thiazole	4-thiazolecarboxylic acid	3973-08-8	1	-0.24	21.7%
thiazole	4-thiazolecarboxylic acid	3973-08-8	50	0.00	0.0%
thiazole	benzothiazole	95-16-9	1	0.00	0.0%
thiazole	benzothiazole	95-16-9	50	-0.44	10.4%
thiazole	thiamine	67-03-8	1	0.00	0.0%
thiazole	thiamine	67-03-8	50	0.00	0.0%
thiazole	thiazole	288-47-1	1	0.00	0.0%
thiazole	thiazole	288-47-1	50	0.00	0.0%
triarylmethane dye	Brilliant Blue FCF	3844-45-9	5	-0.07	3.0%
triarylmethane dye	Brilliant Blue FCF	3844-45-9	50	-0.93	1.0%
triarylmethane dye	Coomassie Brilliant Blue G-250	6104-58-1	5	-2.65	1.4%
triarylmethane dye	Coomassie Brilliant Blue G-250	6104-58-1	50	-2.71	2.0%
triarylmethane dye	Crystal violet	548-62-9	5	-1.47	1.5%
triarylmethane dye	Crystal violet	548-62-9	50	-1.10	0.4%
triarylmethane dye	Erythrosine B	16423-68-0	5	-0.14	3.1%
triarylmethane dye	Erythrosine B	16423-68-0	50	-2.69	5.3%
triarylmethane dye	Fast Green FCF	2353-45-9	5	-0.05	7.1%
triarylmethane dye	Fast Green FCF	2353-45-9	50	-0.18	2.1%
triarylmethane dye	Fluorescein	2321-07-5	5	-2.53	2.2%
triarylmethane dye	Fluorescein	2321-07-5	50	-2.96	1.8%
triarylmethane dye	Malachite green	569-64-2	5	-0.40	0.2%
triarylmethane dye	Malachite green	569-64-2	50	-0.82	3.9%
triarylmethane dye	Rhodamine B	81-88-9	5	-0.35	4.2%
triarylmethane dye	Rhodamine B	81-88-9	50	-2.88	0.8%
triarylmethane dye	phenolphthalein	77-09-8	5	-0.68	2.9%

triarylmethane dye	phenolphthalein	77-09-8	50	-1.04	2.6%

[0097] While aspects of the presently disclosed subject matter have been shown and described, modifications thereof can be made by one skilled in the art without departing from the spirit and teachings of the subject matter. The aspects described herein are exemplary only and are not intended to be limiting. Many variations and modifications of the subject matter disclosed herein are possible and are within the scope of the disclosed subject matter. Where numerical ranges or limitations are expressly stated, about , such express ranges or limitations should be understood to include iterative ranges or limitations of like magnitude falling within the expressly stated ranges or limitations (e.g., from about 1 to about 10 includes, 2, 3, 4, etc.; greater than 0.10 includes 0.11, 0.12, 0.13, about , etc.). Use of the term "optionally" with respect to any element of a claim is intended to mean that the subject element is required, or alternatively, is not required. Both alternatives are intended to be within the scope of the claim. Use of broader terms such as comprises, includes, having, etc. should be understood to provide support for narrower terms such as consisting of, consisting essentially of comprised substantially of, etc.

[0098] Accordingly, the scope of protection is not limited by the description set out above but is only limited by the claims which follow, about , that scope including all equivalents of the subject matter of the claims. Each and every claim is incorporated into the specification as an aspect of the present disclosure. Thus, the claims are a further description and are an addition to the aspects of the presently disclosed subject matter. The discussion of a reference herein is not an admission that it is prior art to the presently disclosed subject matter, especially any reference that may have a publication date after the priority date of this application. The disclosures of all patents, patent applications, and publications cited herein are hereby incorporated by reference, to the extent that they provide exemplary, procedural or other details supplementary to those set forth herein.

CLAIMS

What is claimed is:

1. A catalyst composition, comprising:
an oxidoreductase;
a single electron oxidizer; and
a small molecule activator.

2. The composition of claim 1, wherein the oxidoreductase comprises a copper radical oxidase.

3. The composition of claim 1, wherein the oxidoreductase is selected from the group consisting essentially of a galactose oxidase, a glyoxal oxidase, an alcohol oxidase, mutants thereof and combinations thereof.

4. The composition of claim 1, wherein the oxidoreductase is defined by any of SEQ ID No. 1 through SEQ ID No. 39.

5. The composition of claim 1, wherein the oxidoreductase is present in an amount ranging from about 0.01 g/L to about 1 g/L.

6. The composition of claim 1, wherein the small molecule activator comprises include L-tryptophan, 2-mercaptobenzothiazole, L-histidine, methylchloroisothiazolinone, o-dianisidine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 4-aminoantipyrine, L-tyrosine, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl, chloromethylisothiazolinone, 4-thiazolecarboxylic acid, Sunset yellow FCF, tartrazine, p-benzoquinone, dicoumarol, phthalimide, saccharin, phthalic anhydride, erythrosine B, 2-aminobenzothiazole, thiabendazole, 2-hydroxybenzothiazole, phenothiazine, 6-aminobenzothiazole, indigo carmine, naphthalimide, 2-aminothiazole, thiazole, 2H-1,4-benzothiazin-3(4H)-one, 2-oxindole, beta-lapachone, menaquinone, thiamine, 4-methyl-5-thiazoleethanol, Allura Red AC, menadione, p-cresol, Fast green FCF, Brilliant Blue FCF, methylisothiazolinone, caffeine, veratryl alcohol, fluorescein, or combinations thereof.

7. The composition of claim 1, wherein the small molecule activator is present in an amount ranging from about 1 ppm to about 500 ppm.

8. The composition of claim 1, wherein the single electron oxidizer comprises laccase, horseradish peroxidase, dyp-type peroxidase, lactoperoxidase, chloroperoxidase, manganese peroxidase 1, ascorbate peroxidase, dye-decolorizing peroxidase, unspecific peroxygenase, dehaloperoxidase, catalase-peroxidase, lignin peroxidase, soybean seed coat peroxidase, isoforms thereof or combinations thereof.

9. The composition of claim 1, wherein the single electron oxidizer has any SEQ ID No.45 through SEQ ID No. 62.

10. The composition of claim 1, wherein the single electron oxidizer is present in an amount ranging from about 1 mg/L to about 250 mg/L.

11 The composition of claim 1, further comprising a catalase.

12. The composition of claim 11,wherein the catalase is defined by any SEQ ID No. 40 through SEQ ID No. 44.

13. A method, comprising:
contacting a sugar with a catalyst composition comprising an oxidoreductase, a single electron oxidizer. and a small molecule activator under conditions suitable for the formation of one or more oxidized sugar oxidation products.

14. The method of claim 13, wherein the oxidoreductase is selected from the group consisting essentially of a galactose oxidase, a glyoxal oxidase, an alcohol oxidase, mutants thereof and combinations thereof.

15. The method of claim 13, wherein the oxidoreductase is defined by any of SEQ ID No. 1 through SEQ ID No. 39.

16. The method of claim 13, wherein the small molecule activator comprises include L-tryptophan, 2-mercaptobenzothiazole, L-histidine, methylchloroisothiazolinone, o-dianisidine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 4-aminoantipyrine, L-tyrosine, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl, chloromethylisothiazolinone, 4-thiazolecarboxylic acid, Sunset yellow FCF, tartrazine, p-benzoquinone, dicoumarol, phthalimide, saccharin, phthalic anhydride, erythrosine B, 2-aminobenzothiazole, thiabendazole, 2-hydroxybenzothiazole, phenothiazine, 6-aminobenzothiazole, indigo carmine, naphthalimide, 2-aminothiazole, thiazole, 2H-1,4-benzothiazin-3(4H)-one, 2-oxindole, beta-lapachone, menaquinone, thiamine, 4-methyl-5-thiazoleethanol, Allura Red AC, menadione, p-cresol, Fast green FCF, Brilliant Blue FCF, methylisothiazolinone, caffeine, veratryl alcohol, fluorescein, or combinations thereof.

17. The method of claim 13, wherein the single electron oxidizer comprises laccase, horseradish peroxidase, dyp-type peroxidase, lactoperoxidase, chloroperoxidase, manganese peroxidase 1, ascorbate peroxidase, dye-decolorizing peroxidase, unspecific peroxygenase, dehaloperoxidase, catalase-peroxidase, lignin peroxidase, soybean seed coat peroxidase, isoforms thereof or combinations thereof.

18. The method of claim 13, wherein the single electron oxidizer has any SEQ ID No.45 through SEQ ID No. 62.

19. The method of claim 13, wherein the sugar comprises glucose.

20. The method of claim 13, wherein the one or more sugar oxidation products comprise aldonic acid, uronic acid, aldaric acid, a gluconic acid oxidation product, a gluconate, glucaric acid, gluconic acid, glucuronic acid, glucose oxidation products, galactonic acid, galactaric acid, glutamic acid, a lactone of gluconic acid, a lactone of glucaric acid, a lactone of galactaric acid, a lactone of galactonic acid, glucodialdose, 2-ketoglucose, disaccharides, oxidized disaccharides, n-keto-acids, C2 to C6 diacids, salts thereof or combinations thereof.

Enz Enz His' Cu II O⁻ H O⁻ His O H C H R S Enz PT H O H His' Cu II O His - O + H C H R S HAT or PCET ET

FIG. 1A

O_2 ET HAT or PCET PT O_2H_2

FIG. 1B

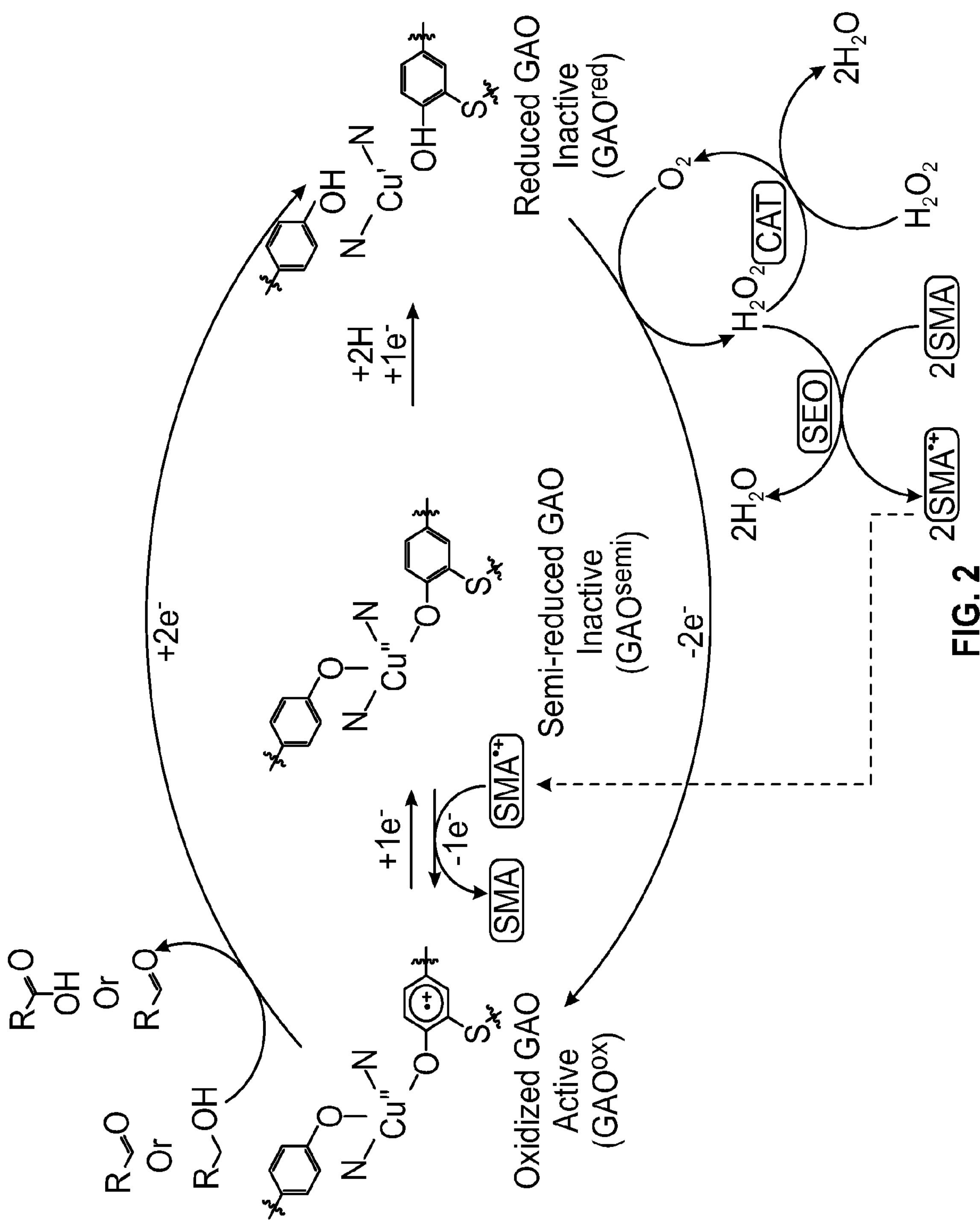


FIG. 2

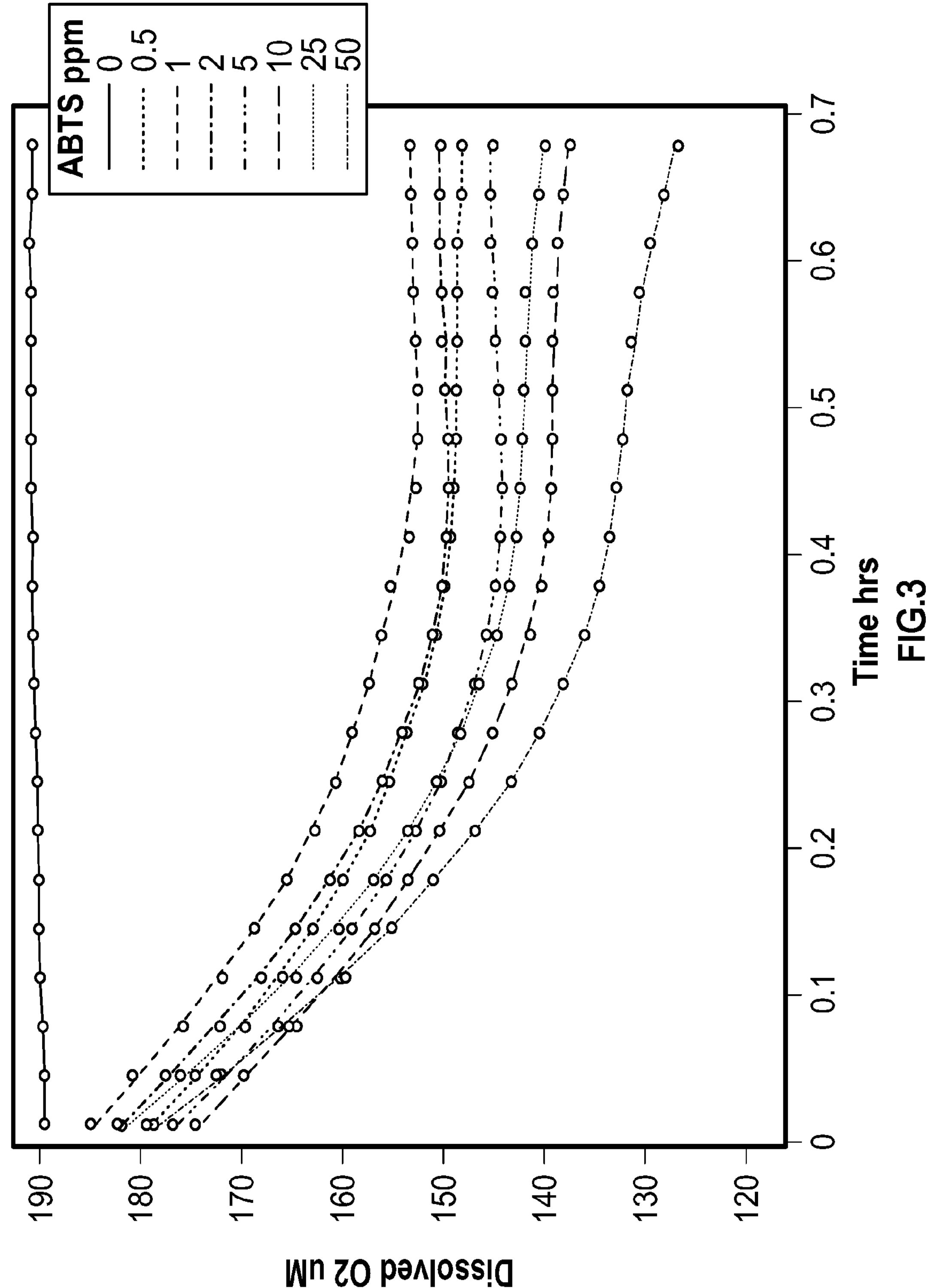


FIG.3

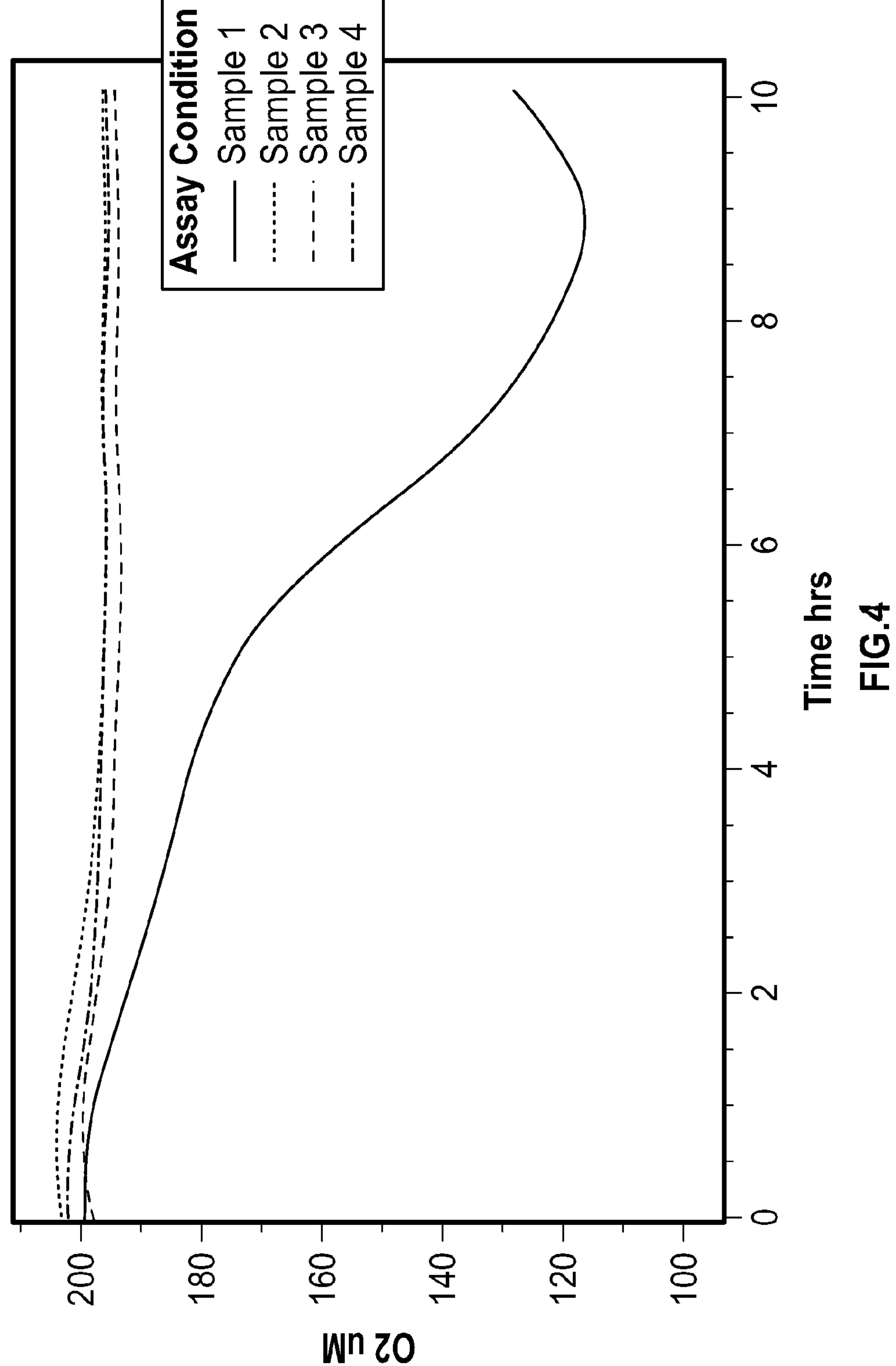
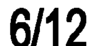


FIG.4

FIG.5



Conversion After 24 Hours of Reaction

60
50
40
30
20
10
0

0.005 g/L HRP
0.5 g/LDtpA
1 g/L DtpA

FIG.6

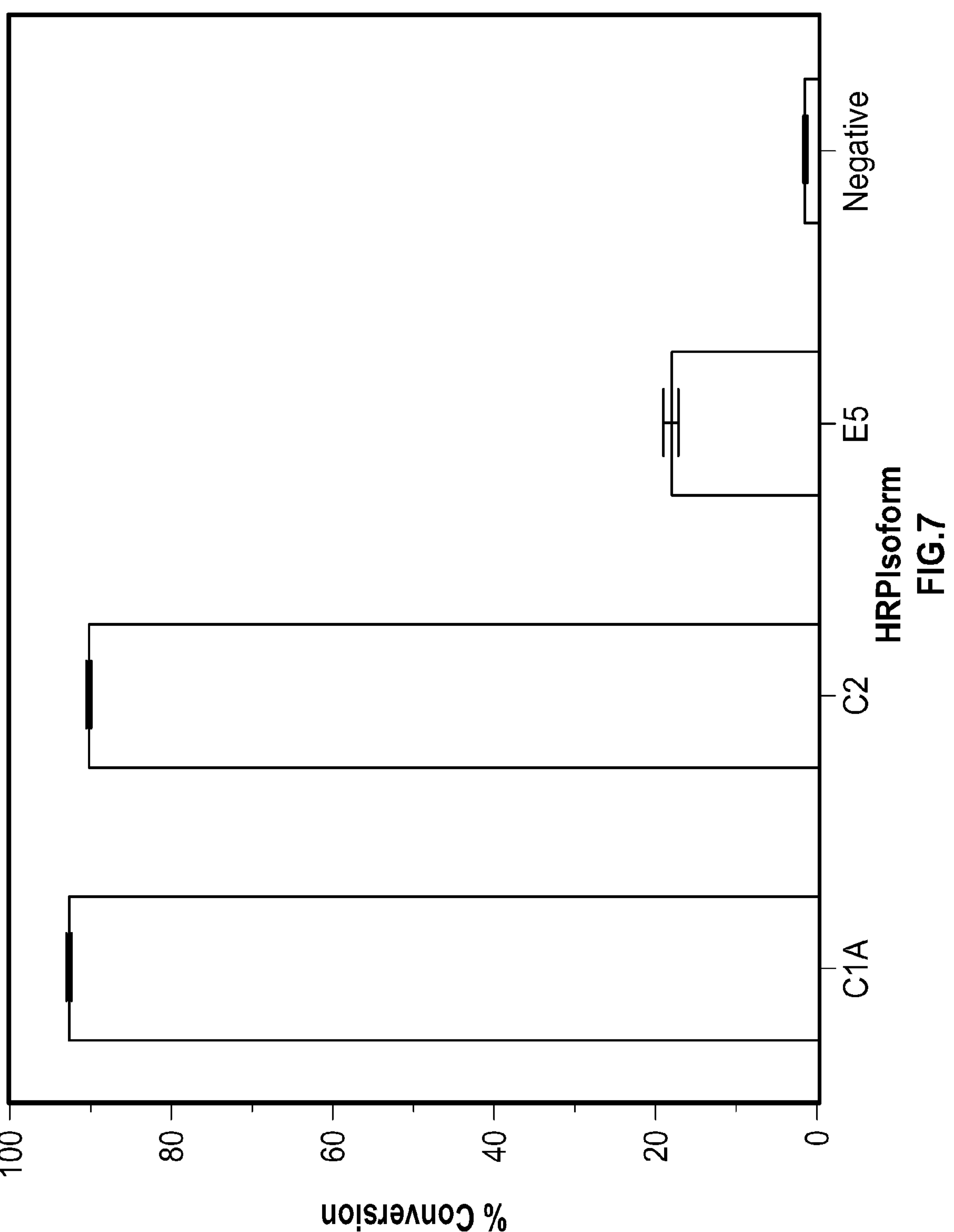


FIG.7

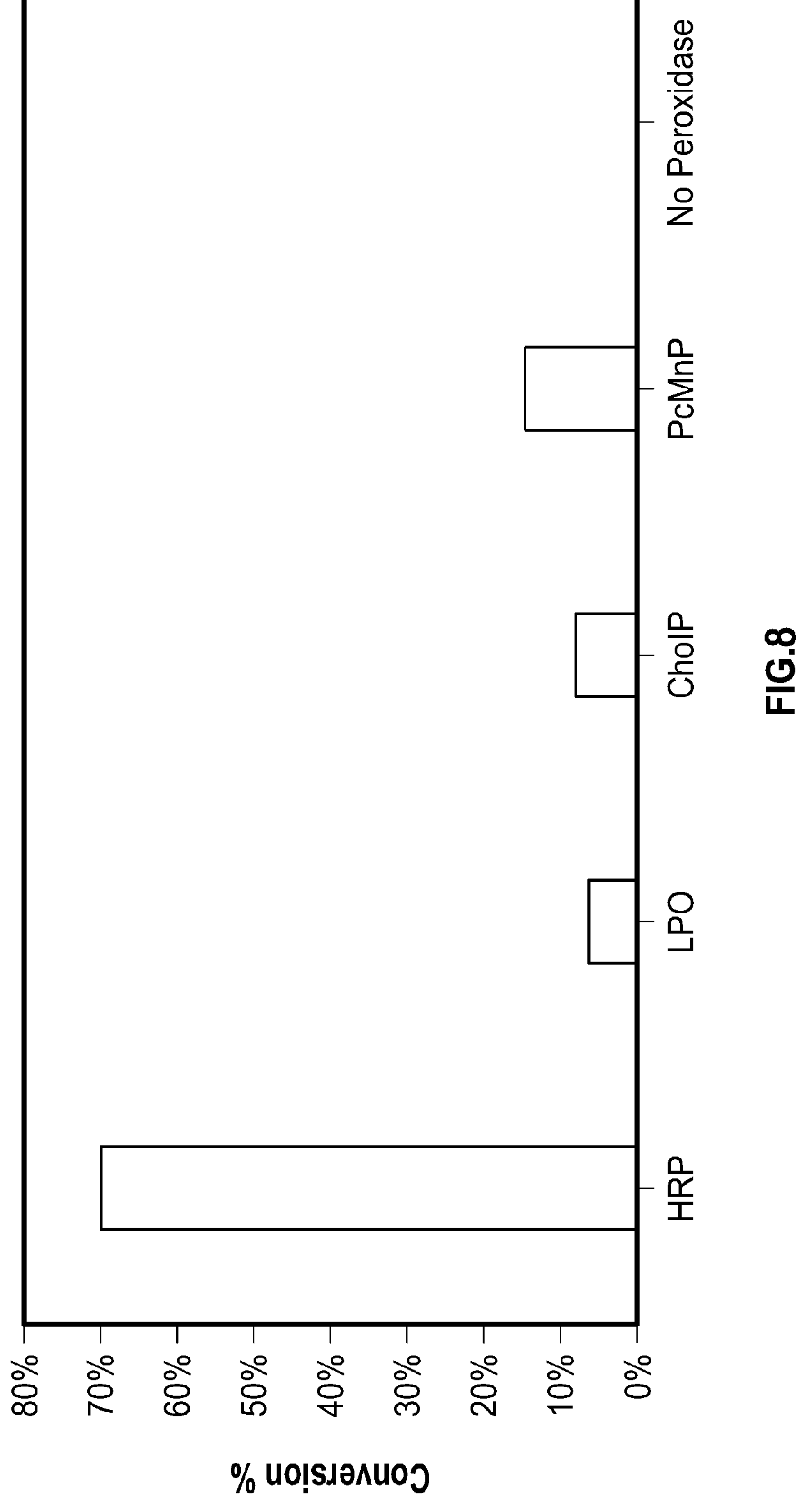


FIG.8

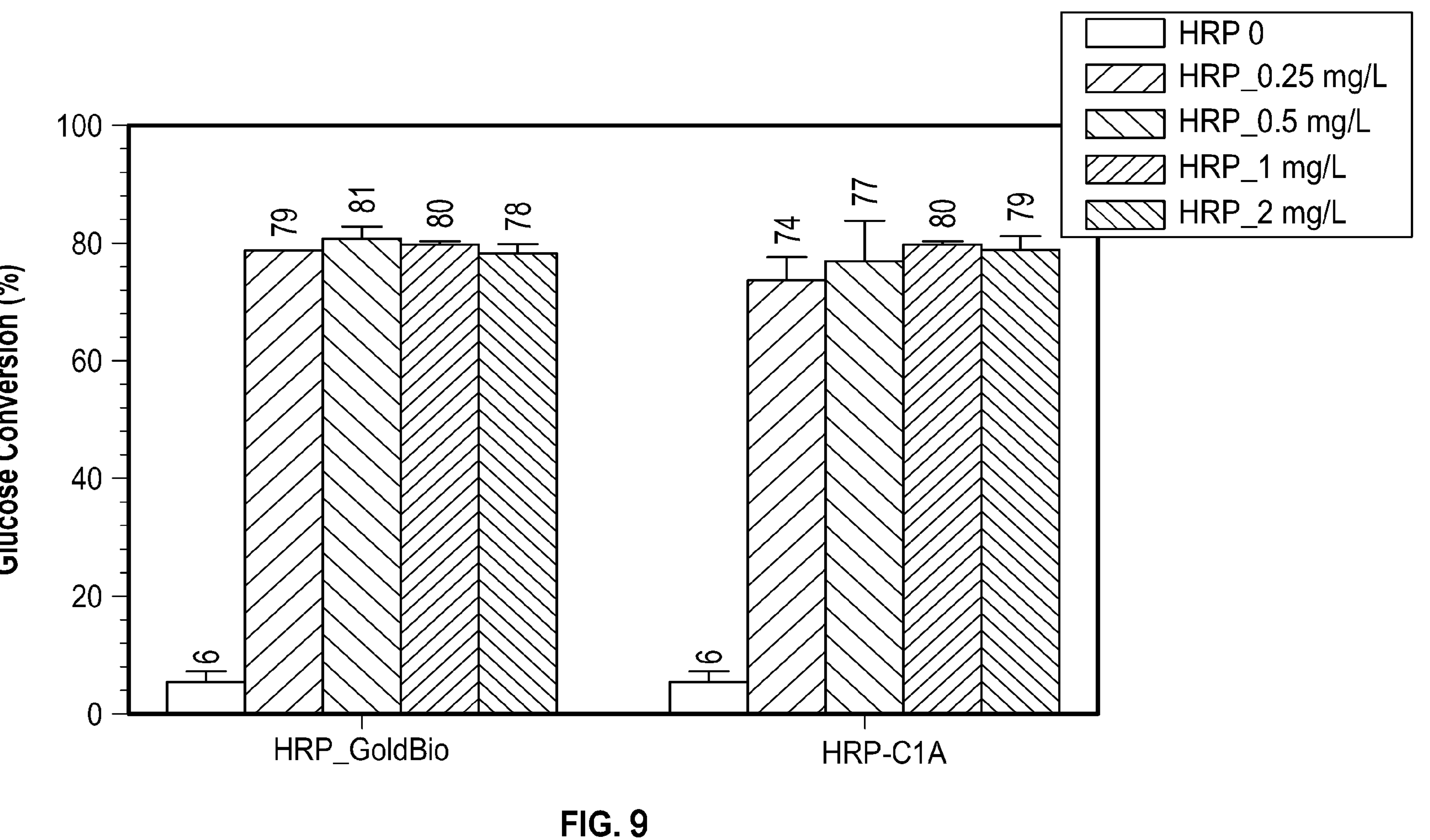


FIG. 9

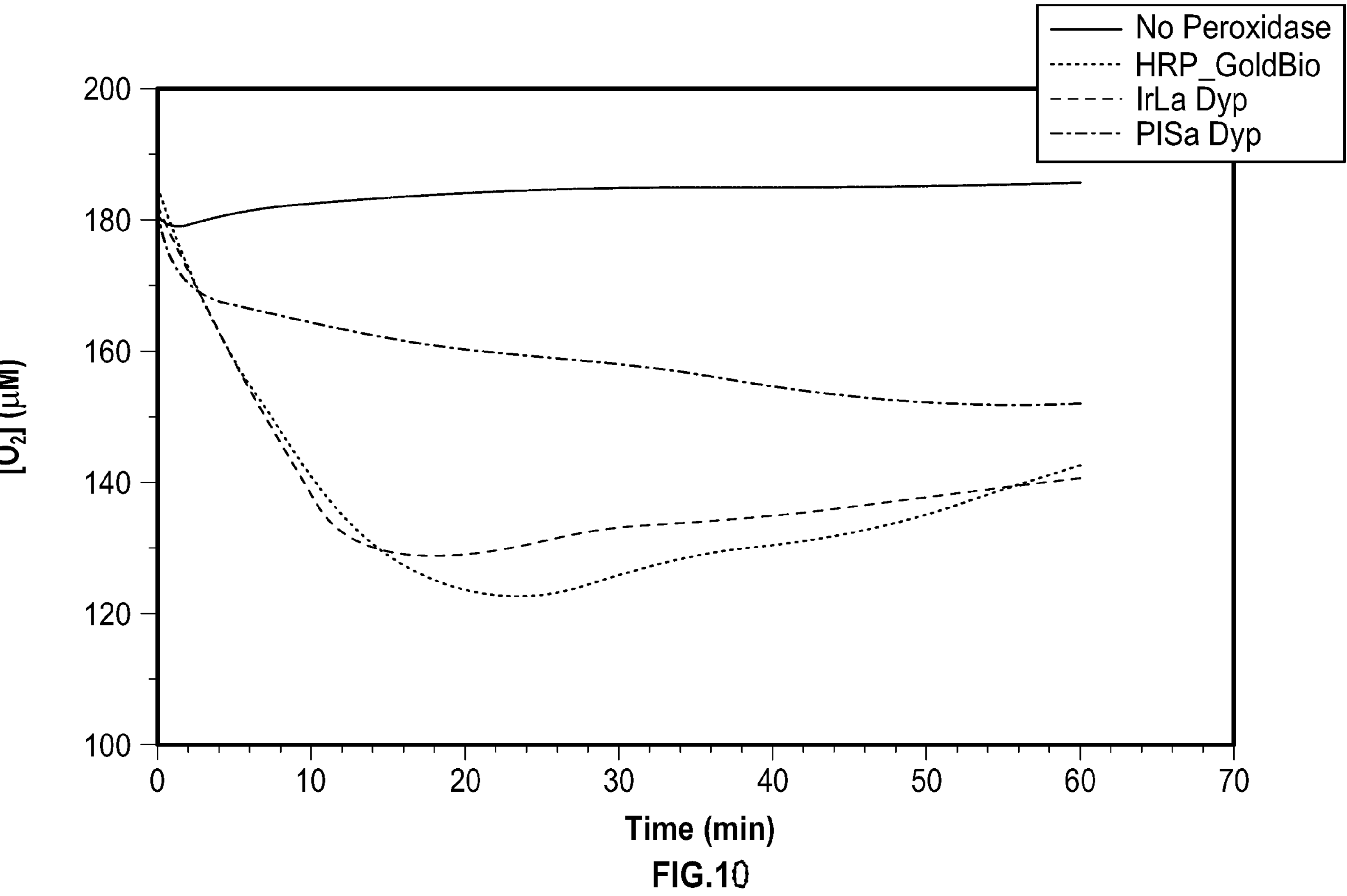


FIG.10

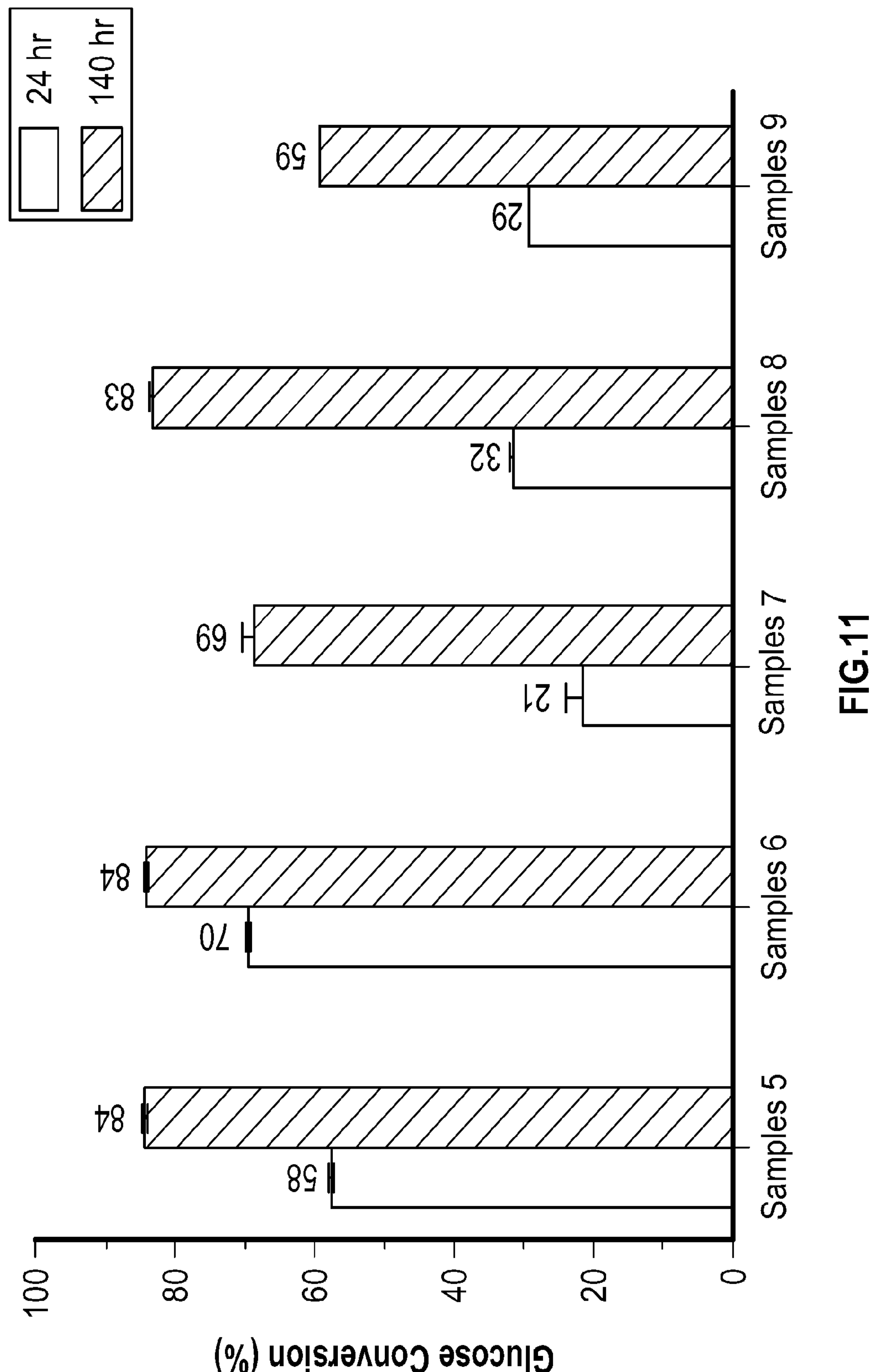


FIG.11

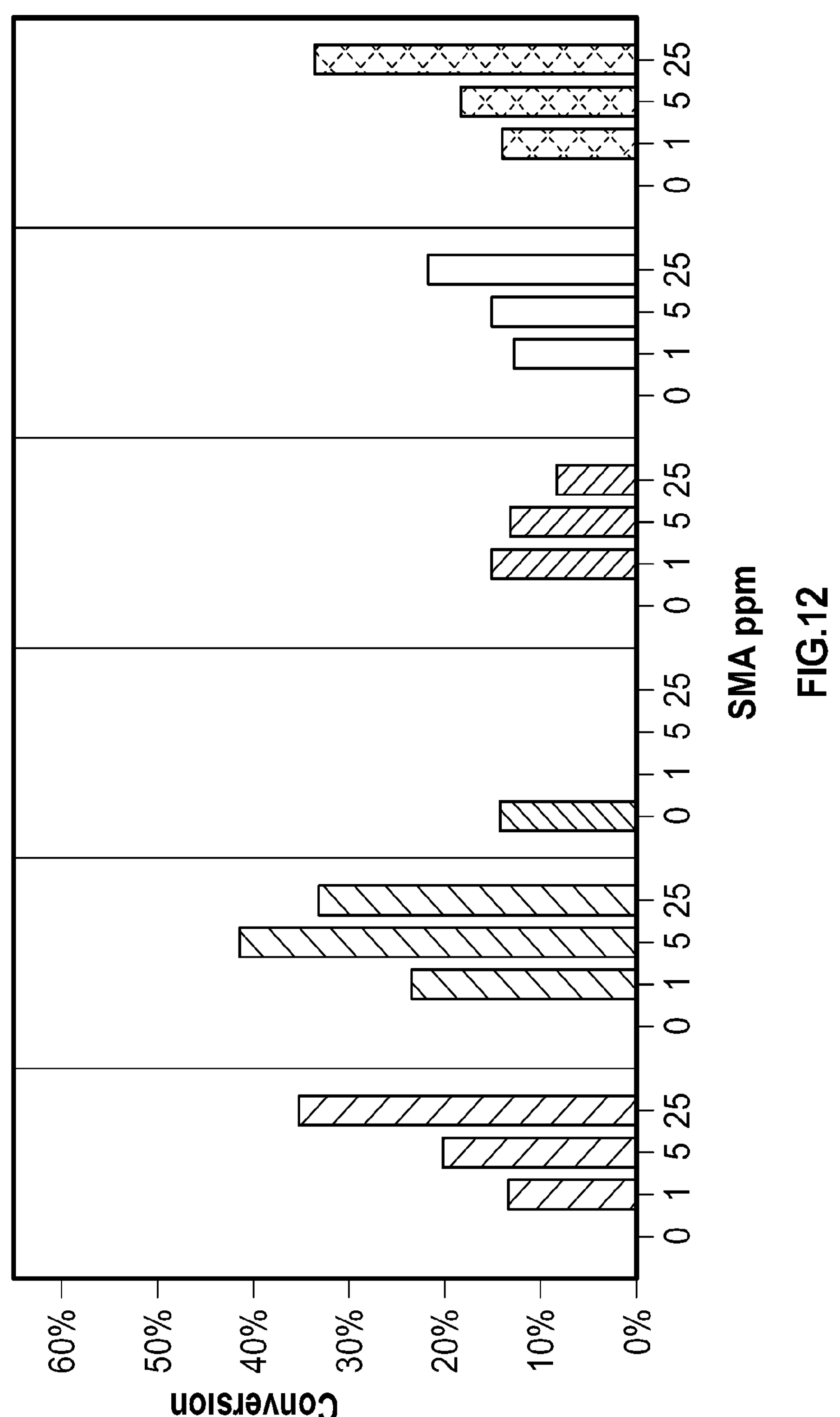


FIG.12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/050277

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***B01J 8/00*** (2024.01); ***B01J 23/72*** (2024.01); ***C01B 15/01*** (2024.01)

CPC: ***B01J8/00; B01J23/72; C01B15/01;*** *C12Y101/03004; C12Y101/03009*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	WO 2021/178935 A1 (SOLUGEN INC.) 10 September 2021 (10.09.2021)	
X	[0007], [0014], [0048], [0049], [0063], [0066], [0092], [00167], [00170], [00178], [00179] ; Abstract	1-3, 8, 10-11, 13-14, 17, 19-20
Y		5, 6, 7, 12, 16
A		9 and 18
	WO 2023/004432 A2 (SOLUGEN INC.) 26 January 2023 (26.01.2023)	
X	[0096], [00104], [00171], [00197], [00209],	1, 4, 13 and 15
	US 2014/0107377 A1 (THE PROCTER & GAMBLE COMPANY) 17 April 2014 (17.04.2014)	
Y	[0092]	12
	WO 2016/207343 A1 (TECHNISCHE UNIVERSITAT GRAZ) 29 December 2016 (29.12.2016)	
A	[0058]	9 and 18

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 2024 (11.12.2024)	**24 March 2025 (24.03.2025)**

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/050277

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,902,918 B1 (ARNOLD FRANCES H) 07 June 2005 (07.06.2005) Fig.23	9 and 18
P,X	WO 2024/129887 A1 (SOLUGEN INC.) 20 June 2024 (20.06.2024) Entire document	1-3, 6, 8, 11, 13-14, 17, 19-20
P,A		4-5, 7, 9-10, 12, 15-16, 18
Y	WU. "Biocatalysis: Enzymatic Synthesis for Industrial Applications" 88-119. Enzyme Catalysis. Web. 22 December 2020; <DOI: 10.1002/anie.202006648> Page 98	5
Y	BEGAM. "Adsorption isotherm and kinetic studies for the decolorization of sunset yellow FCF dye using economically feasible low-cost adsorbent" 276-285. Global Nest. Web. 28 March 2022; <DOI: 10.30955/gnj.004266> Abstract	6-7 and 16

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☐ forming part of the international application as filed.
   b. ☑ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☑ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: claims 1-20, SEQ ID NO: 1 (oxidoreductase sequence), SEQ ID NO: 45 (single electron oxidizer sequence), SEQ ID NO: 40 (catalase sequence), are directed to a catalyst composition for formation of oxidized sugar oxidation products.

The methods of Claims 1-3, 4 (in part), 5-8, 9 (in part), 10-11, 12 (in part), 13-14, 15 (in part), 16-17, 18 (in part), 19-20 are believed to encompass the first named invention of Groups I+ and are the claims that will be searched to the extent that they comprise a composition encompassing SEQ ID NO: 1 (first exemplary oxidoreductase sequence), SEQ ID NO: 45 (first exemplary single electron oxidizer sequence), SEQ ID NO: 40 (first exemplary catalase sequence). This first named invention of Group I+ has been selected to encompass the first species of each of the genera found in claims 4, 9, 12, 15, are based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines.

Applicant is invited to elect additional sequences, where available as an option within at least one searchable claim, to be searched. Additional sequence(s) will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 2 (oxidoreductase sequence), SEQ ID NO: 46 (single electron oxidizer sequence), SEQ ID NO: 41 (catalase sequence)

Groups I+ share the technical features including: a catalyst composition, comprising: an oxidoreductase; a single electron oxidizer; and a small molecule activator; a method, comprising: contacting a sugar with a catalyst composition comprising an oxidoreductase, a single electron oxidizer. and a small molecule activator under conditions suitable for the formation of one or more oxidized sugar oxidation products.

However, these shared technical features are previously disclosed by WO 2021/178935 A1 to Solugen, Inc. (hereinafter "Solugen").

Solugen discloses a catalyst composition (catalyst oxidation; [0007]), comprising: an oxidoreductase (oxidoreductase; [0066]); a single electron oxidizer (horseradish peroxidase used in method; [0049]); and a small molecule activator (tyrosyl radical included in catalysis reaction; [0063]); a method, comprising: contacting a sugar with a catalyst composition (sugar contacted with composition; abstract) comprising an oxidoreductase (oxidoreductase; [0066]), a single electron oxidizer (horseradish peroxidase used in method; [0049]). and a small molecule activator under conditions (tyrosyl radical (small molecule activator) included in catalysis reaction; [0063]) suitable for the formation of one or more oxidized sugar oxidation products (production of glucose oxidation products; abstract).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Solugen reference, unity of invention is lacking.

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-20, SEQ ID NO: 1 (oxidoreductase sequence), SEQ ID NO: 45 (single electron oxidizer sequence), SEQ ID NO: 40 (catalase sequence).;**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.