(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 December 2025 (18.12.2025)

WIPO | PCT

(10) International Publication Number
WO 2025/259650 A1

(51) International Patent Classification:
C12P 7/02 (2006.01) *C12P 7/40* (2006.01)
C12P 7/24 (2006.01) *B01J 23/66* (2006.01)

(21) International Application Number:
PCT/US2025/032974

(22) International Filing Date:
10 June 2025 (10.06.2025)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/658,207 10 June 2024 (10.06.2024) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UY, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST,

(54) Title: COMPOSITIONS AND METHODS FOR GLYCOLIC ACID PRODUCTION

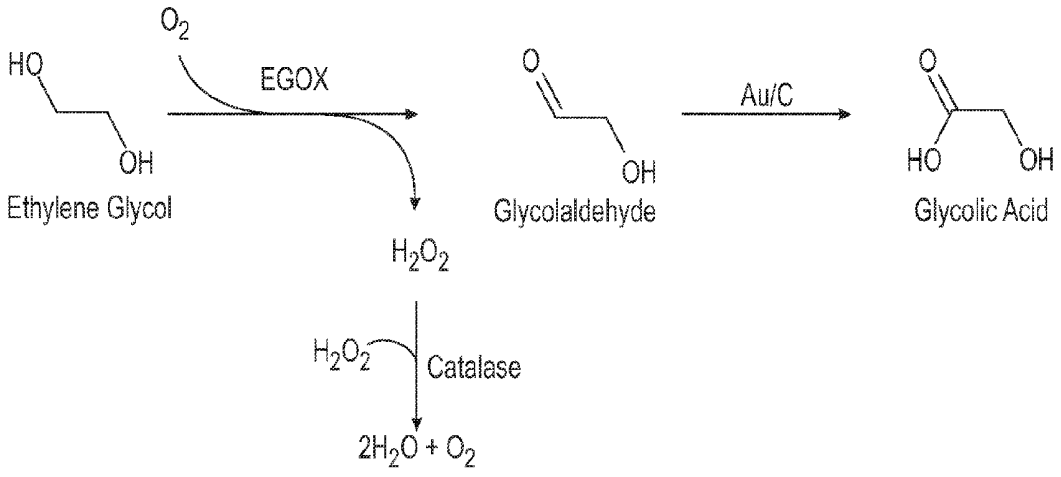

FIG. 1

(57) Abstract: A method for the production of glycolic acid comprising contacting ethylene glycol with an oxidase catalyst system under conditions suitable for the formation of glycolaldehyde; contacting glycolaldehyde with one or more metal oxidation catalysts under conditions suitable for the formation of glycolic acid; and recovering at least a portion of the glycolic acid. A method for the production of glycolic acid comprising contacting ethylene glycol with an oxidase catalyst system comprising galactose oxidase under conditions suitable for the formation of glycolaldehyde; contacting glycolaldehyde with an oxidation catalyst comprising gold on a carbon support under conditions suitable for the formation of glycolic acid; and recovering at least a portion of the glycolic acid.

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SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

COMPOSITIONS AND METHODS FOR GLYCOLIC ACID PRODUCTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit and priority of U.S. provisional patent application Serial No. 63/658,207 filed June 10, 2024, and entitled "COMPOSITIONS AND METHODS FOR GLYCOLIC ACID PRODUCTION," which is hereby incorporated herein by reference in its entirety for all purposes.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

REFERENCE TO SEQUENCE LISTING

[0003] 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 June 9, 2025 is named "23ENZ007-PCT_3416-01805.xml" and is 17,270 bytes in size.

TECHNICAL FIELD

[0004] The present disclosure relates generally to the production of higher value chemicals. More particularly, the present disclosure relates generally higher value chemicals derived from sugar oxidation products. Still more particularly, the present disclosure relates to novel methods for the production of glycolic acid.

BACKGROUND

[0005] With sustainability being a desired goal, tremendous progress in bio-based production routes from renewable raw materials to commercial goods continues to occur. Of particular interest is the formation of higher value chemicals from what are termed "platform molecules." Herein platform molecules refer to bio-based or bio-derived chemicals whose constituting elements totally originated from biomass and could be used as building blocks for the generation of commodity and refined chemicals.

[0006] Glycolic acid (hydroacetic acid or hydroxyacetic acid); chemical formula $C_2H_4O_3$ (also written as $HOCH_2CO_2H$), is the smallest α-hydroxy acid (AHA). This colorless, odorless, and hygroscopic crystalline solid is highly soluble in water. Glycolic acid is used in a plethora of industries due to its bactericidal and chelating properties. Glycolic acid's ability to form a chelate with calcium(II) ions is exploited in the leather industry to

delime hides, in alum and chrome mordants, and in fur-processing operations. Glycolic acid may also serve as a complexing agent for other metals with applications in copper polishes, etching agents for lithographic plates, and electropolishing and galvanizing baths. The bactericidal properties of glycolic acid make the compound suitable for incorporation into acidic cleansing agents, especially in cleaning operations of milk storage and production equipment, drinking fountains, and rust and scale removal in heat exchangers and pipelines. More recently, glycolic acid has enjoyed use in the cosmetics, polymer degradable materials (particularly in the synthesis of biodegradable polyglycolic acid (PGA), and drug production industries.

[0007] Methods for the industrial production of glycolic acid have included (i) treatment of formaldehyde or trioxymethylene with carbon monoxide and water in the presence of an acid catalyst under high pressures (e.g., greater than 30 MPa); (ii) electrolytic reduction of oxalic acid to form glycolic acid; and (iii) hydrolysis of the nitrile of oxalic acid. All of these processes involve the use of hazardous chemicals such as formaldehyde, carbon monoxide, or hydrocyanic acid and feedstocks derived from fossil fuels.

[0008] Ethylene glycol (EG) is a highly promising substrate for orthogonal production of a variety of chemicals because it minimized the interactions between biomass and chemical producing pathways. An ongoing need exists for novel methods and compositions for production of high purity glycolic acid that addresses one or more of the aforementioned challenges.

BRIEF SUMMARY OF THE DISCLOSURE

[0009] Disclosed herein is a method for the production of glycolic acid comprising contacting ethylene glycol with an oxidase catalyst system under conditions suitable for the formation of glycolaldehyde; contacting glycolaldehyde with one or more metal oxidation catalysts under conditions suitable for the formation of glycolic acid; and recovering at least a portion of the glycolic acid.

[0010] Also disclosed herein is a method for the production of glycolic acid comprising contacting ethylene glycol with an oxidase catalyst system comprising galactose oxidase under conditions suitable for the formation of glycolaldehyde; contacting glycolaldehyde with an oxidation catalyst comprising gold on a carbon support under conditions suitable for the formation of glycolic acid; and recovering at least a portion of the glycolic acid.

[0011] Aspects described herein comprise a combination of features and characteristics intended to address various shortcomings associated with certain prior devices, systems, and methods. The foregoing has outlined rather broadly the features and technical characteristics of the disclosed aspects in order that the detailed description that follows may be better understood. The various characteristics and features described above, as well as others, will be readily apparent to those skilled in the art upon reading the following detailed description, and by referring to the accompanying drawings. It should be appreciated that the conception and the specific aspects disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes as the disclosed aspects. It should also be realized that such equivalent constructions do not depart from the spirit and scope of the principles disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] For a detailed description of various exemplary aspects, reference will now be made to the accompanying drawings in which:

[0013] Figure 1 depicts schematically the reaction of ethylene glycol and an oxidase catalyst system to produce an intermediate that is subsequently oxidized by a metal catalyst to form glycolic acid.

[0014] Figure 2 depicts schematically the reaction of ethylene glycol and two oxidase catalyst systems to produce glycolic acid.

[0015] Figure 3 depicts schematically a reactor system for production of glycolic acid.

DETAILED DESCRIPTION

[0016] The following discussion is directed to various exemplary aspects. However, one of ordinary skill in the art will understand that the examples disclosed herein have broad application, and that the discussion of any aspect is meant only to be exemplary of that aspect, and not intended to suggest that the scope of the disclosure, including the claims, is limited to that aspect.

[0017] The figures are not necessarily to scale. Certain features and components herein may be shown exaggerated in scale or in somewhat schematic form and some details of conventional elements may not be shown in interest of clarity and conciseness.

[0018] In the following discussion and in the claims, the terms "including" and "comprising" are used in an open-ended fashion, and thus should be interpreted to mean "including, but not limited to... ." As used herein, the terms "approximately," "about,"

"substantially," and the like mean within 10% (i.e., plus or minus 10%) of the recited value. Thus, for example, a recited angle of "about 80 degrees" refers to an angle ranging from 72 degrees to 88 degrees.

[0019] Disclosed herein are compositions and methods for the chemoenzymatic production of glycolic acid. Also disclosed herein are compositions and methods for the enzymatic production of glycolic acid. In one or more aspects, a method of the present disclosure comprises contacting ethylene glycol with a biocatalyst under conditions suitable for the formation of an aldehyde intermediate. Hereinafter this is referred to as "Stage I" of the method. In one or more aspects, a method of the present disclosure further comprises converting the aldehyde intermediated to the corresponding hydrocarboxylic acid in the presence of a metal catalyst. Hereinafter this is referred to as "Stage 2" of the method.

[0020] It is to be understood that the methods described herein may be numerically ordered in stages (i.e., Stage 1, Stage 2) for ease of reference however it is not intended to limit the performance of the activities in each stage to a particular order. For example, one or more activities described for a particular stage may be carried out concurrently with one or more activities of another stage whether that "another stage" is designated numerically as being subsequent to or prior to the "particular stage." Such modifications in terms of the timing of the activities performed in any particular stage may be made by one of ordinary skill in the art with the benefits of the present disclosure.

[0021] With reference to Figure 1, a chemoenzymatic method of the present disclosure comprises contacting ethylene glycol with one or more oxidase catalyst systems under conditions suitable to produce one or more oxidized ethylene glycol products. Herein an oxidase catalyst system comprises (a) one or more oxidases; and optionally (b) one or more cofactors; each of which are described in further detail herein.

[0022] In an aspect, any enzyme that can catalyze the oxidation of ethylene glycol to glycolaldehyde, under the conditions disclosed herein, is suitable for use in the present disclosure. In another aspect, any enzyme that can catalyze the oxidation of glycolaldehyde under the conditions disclosed herein, is suitable for use in the methods of the present disclosure. In the alternative, the oxidase catalyst system comprises a copper radical oxidase such as a galactose oxidase, an alcohol oxidase, a glycerol oxidase, an unspecific peroxygenase or combinations thereof.

[0023] In an aspect, the oxidase catalyst system comprises a galactose oxidase (GAO). GAO is a copper enzyme secreted by some fungal species, particularly *Fusarium*

graminearum (also known as *Gibberella zeae*), that aids in the degradation of extracellular carbohydrate food sources through catalyzing the oxidation of primary alcohols to aldehydes while generating oxygen and hydrogen peroxide. The native function of GAO is the oxidation of D-galactose to D-galacto-hexodialdose.

[0024] In one or more aspects, the oxidase catalyst system comprises an alcohol oxidase (AOX). Highly characterized alcohol oxidases are available as catalysts. For example, FAD-bound AOX from methylotrophic yeast is capable of generating glycolaldehyde and glyoxal from ethylene glycol. In one or more aspects, the oxidase catalyst system comprises a glycerol oxidase (GLOX).

[0025] In one or more aspects, the oxidase catalyst system comprises an unspecific peroxygenase. Unspecific peroxygenase (UPO), also known as aromatic peroxygenase (APO), are largely extracellular heme-thiolate proteins found in fungal species that utilize hydrogen peroxide to transfer an oxygen atom to a substrate. The first UPO enzyme was discovered in in *Agrocybe aegerita* (AaeUPO), the Black Poplar mushroom. Since then, other fungal UPOs have been characterized from *Marasmius rotula* (MroUPO), *Coprinellus radians* or *Coprinus radians* (CraUPO), *Chaetomium globosum* (CglUPO), *Sulfurisphaera tokodaii* (StoUPO), *Collariella viriscens* or *Chaetomium viriscens* (CviUPO), *Daldinia caldariorum* (DcaUPO), *Marasmius wettsteinii* (MweUPO), *Coprinopsis cinerea* (CciUPO), and *Humicola insolens* (HinUPO).

[0026] In one or more aspects, the oxidase catalyst system comprises an oxidase having any of SEQ ID NO:1 through SEQ ID NO:11.

[0027] The oxidase catalyst systems disclosed herein may be utilized under reaction conditions comprising one or more of the following parameters: an amount of reactant (e.g., ethylene glycol) of from about 0.1 weight per volume percent (w/v%) to about 60 w/v%; additionally or alternatively, from about 5 w/v% to about 50 w/v%; additionally or alternatively, from about 10 w/v% to about 40 w/v% and an amount of oxidase (e.g., GAO) of from about 0.1 mg/L to about 30,000 mg/L; additionally or alternatively, from about 5 mg/L to about 500 mg/L; additionally or alternatively, from about 10 mg/L about 100 mg/L based on desired throughput of the reaction; and an oxygen pressure of from about 10 psi to about 400 psi; additionally or alternatively from about 25 psi to 300 psi; additionally or alternatively from about 50 psi to about 200 psi. Further reaction conditions may include a temperature ranging from about 1 °C to about 70 °C, additionally or alternatively from about 5 °C to about 30 °C; additionally or alternatively

from about 10 °C to about 25 °C and an aqueous media such as a phosphate buffer at a pH of from about 5 to about 10; additionally or alternatively from about 6 to about 9; additionally or alternatively from about 7 to about 8.5.

[0028] In one or more aspects, the oxidase catalyst systems disclosed herein comprise an oxidase or a mutated oxidase which is present 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.

[0029] In some aspects, the oxidase catalyst system comprises an optional cofactor, an optional small molecule activator (SMA), an optional single electron oxidizer (SEO) or combinations thereof.

[0030] In some aspects the oxidase catalyst system comprises an SEO 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 and combinations thereof.

[0031] In some aspects the oxidase catalyst system comprises an SMA such as 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

[0032] In some aspects the oxidase catalyst system comprises a cofactor such as thiamine pyrophosphate, NAD+, NADP+, pyridoxal phosphate, methyl cobalamin,

cobalamine, biotin, Coenzyme A, tetrahydrofolic acid, menaquinone, ascorbic acid, flavin mononucleotide, flavin adenine dinucleotide, and Coenzyme F420.

[0033] The optional cofactors disclosed may be present individually 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; additionally or alternatively, 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.

[0034] As will be understood by one of ordinary skill in the art with the benefit of the present disclosure, reactions of the type disclosed herein may result in the production of byproducts (e.g., hydrogen peroxide, etc.) that can detrimentally impact other components of the reaction mixture. For example, hydrogen peroxide may degrade the oxidase resulting in a loss of catalytic activity. In such aspects, mitigation of the detrimental effects of hydrogen peroxide may be carried out such as by the introduction

of a catalase (E.C. 1.11.1.61), the use of a hydrogen peroxide-resistant enzyme or combinations thereof.

[0035] In an aspect, any enzyme of the type disclosed herein is a wild type enzyme, a functional fragment thereof, or a functional variant thereof. “Fragment” as used herein is meant to include any amino acid sequence shorter than the full-length enzyme, but where the fragment maintains a catalytic activity sufficient to meet some user or process goal. Fragments may include a single contiguous sequence identical to a portion of the biocatalyst sequence. Alternatively, the fragment may have or include several different shorter segments where each segment is identical in amino acid sequence to a different portion of the amino acid sequence of the enzyme but linked via amino acids differing in sequence from the enzyme. Herein, a "functional variant" of the enzyme refers to a polypeptide which has at one or more positions of an amino acid insertion, deletion, or substitution, either conservative or non-conservative, and wherein each of these types of changes may occur alone, or in combination with one or more of the others, and/or one or more times in a given sequence but retains catalytic activity.

[0036] In the alternative or in combination with the aforementioned mutations, the enzyme may be mutated to improve the catalytic activity. Mutations may be carried out to enhance the protein or a homolog activity, increase the protein stability in the presence of substrates and products (e.g., hydrogen peroxide) and increase protein yield.

[0037] 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.

[0038] In an aspect, any 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.

[0039] 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 with the gene of interest 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.

[0040] The term "expression control sequence" 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, etc.); 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 sequences" is intended to include, at a minimum, all components whose presence is essential for expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0041] 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.

[0042] In an aspect, the products of the reaction of ethylene glycol with an oxidase catalyst system (e.g., EGOX, optional cofactors, oxygen) under conditions of the type disclosed herein, include glycolaldehyde. The glycolaldehyde may be purified and/or isolated from any other reaction products using any suitable methodology (e.g., chromatography, distillation, evaporation) to produce a glycolaldehyde that meets one or more user and/or process goals. The glycolaldehyde may be used as the reactant in Stage 2 of the disclosed methodology. For example, glycolaldehyde can be processed to obtain a purity of from about 70% to about 99%, additionally or alternatively from about 75% to about 90%, additionally or alternatively from about 80% to about 90%. Alternatively, the products of the reaction, are used without further processing.

[0043] In an aspect, a method of the present disclosure comprises a second stage, Stage 2, wherein an intermediate (e.g., glycolaldehyde) is contacted with one or more metal catalysts under conditions suitable to produce glycolic acid. For example, Stage 2 may involve converting the aldehyde intermediate (e.g., glycolaldehyde) into a carboxylic acid (e.g., glycolic acid). In one or more aspects, conversion of the aldehyde intermediate to the corresponding carboxylic acid is carried out in the presence of metal oxidation catalyst (MOC). In one or more aspects, the MOC comprises (a) a metal and (b) a support.

[0044] A support suitable for use in the MOC can have a surface area of from about 100 m^2/g to about 1000 m^2/g, additionally or alternatively from about 200 m^2/g to about 900 m^2/g; additionally or alternatively from about 250 m^2/g to about 500 m^2/g; additionally or alternatively about 100 m^2/g, about 125 m^2/g, about 150 m^2/g, about 175 m^2/g, about 200 m^2/g, about 225 m^2/g, about 250 m^2/g, about 275 m^2/g, about 300 m^2/g, about 325 m^2/g, about 350 m^2/g, about 375 m^2/g, about 400 m^2/g g, about 425 m^2/g, about 450 m^2/g, about 475 m^2/g, about 500 m^2/g, about 525 m^2/g, about 550 m^2/g, about 575 m^2/g, about 600 m^2/g, about 625 m^2/g, about 650 m^2/g, about 675 m^2/g, about 700 m^2/g, about 725 m^2/g, about 750 m^2/g, about 775 m^2/g, about 800 m^2/g, about 825 m^2/g, about 850 m^2/g, about 875 m^2/g, about 900 m^2/g, about 925 m^2/g, about 950 m^2/g, about 975 m^2/g, or about 1000 m^2/g.

[0045] In one or more aspects, the support is characterized by a pore volume of from about 0.10 cc/g to about 0.4 cc/g; additionally or alternatively from about 0.10 g/cc to about 0.3 g/cc; additionally or alternatively from about 0.1 g/cc to about 0.25 g/cc;

additionally or alternatively about 0.1 g/cc, about 0.12 g/cc, about 0.14 g/cc, about 0.16 g/cc, about 0.18 g/cc, about 0.2 g/cc, about 0.22 g/cc, about 0.24 g/cc, about 0.26 g/cc, about 0.28 g/cc, about 0.3 g/cc, about 0.32 g/cc, about 0.34 g/cc, about 0.36 g/cc, about 0.38 or about 0.4 g/cc.

[0046] In one or more aspects, the support is characterized by a pore size of from about 0.5 nm to about 5 nm; additionally from about 1 nm to about 5 nm; additionally or alternatively from about 2.5 nm to about 5 nm; additionally or alternatively about 0.5 nm, about 0.6 nm, about 0.8 nm, about 1 nm, about 1.2 nm, about 1.4 nm, about 1.6 nm, about 1.8 nm, about 2 nm, about 2.2 nm, about 2.4 nm, about 2.6 nm, about 2.8 nm, about 3 nm, about 3.2 nm, about 3.4 nm, about 3.6 nm, about 3.8 nm, about 4 nm, about 4.2 nm, about 4.4 nm, about 4.6 nm, about 4.8 nm, or about 5 nm.

[0047] In one or more aspects, the support comprises carbon, ceramic, or metal oxides. In an aspect, the MOC comprises carbon, titania (TiO_2), zirconia (ZrO_2) or any combination thereof which contain less than about 1 weight percent (wt.%), alternatively less than about 0.1 weight percent (wt.%) or alternatively less than about 0.01 wt.% SiO_2 binders based on the total weight of the support. In one or more aspects, the support material is predominantly mesoporous or macroporous and substantially free from micropores. For example, the support may comprise less than about 20% micropores, alternatively less than about 10% micropores, alternatively less than about 5% micropores, alternatively less than about 2.5% micropores, alternatively less than about 1% micropores or alternatively less than about 0.5% micropores.

[0048] In an aspect, a support material for use in the present disclosure is characterized by mesopores having a pore size ranging from about 10 nm to about 100 nm; and a surface area ranging from greater than about 20 $m^2 g^{-1}$ to less than about 300 $m^2 g^{-1}$. Supports suitable for use in the present disclosure may have any suitable shape. For example, the support may be shaped into 0.8-3 mm trilobes, quadralobes, or pellet extrudates. The MOC can be shaped by any suitable methodology such as by extrusion or tableting. For example, the MOC can be shaped to facilitate utilization of the catalyst in reactors such as fluidized, moving bed or fixed bed reaction type with or without continuous flow thereby allowing a broad flexibility regarding the adjustment of the process conditions.

[0049] In one or more aspects, the MOC comprises one or more metals or one or more noble metals such as gold, silver, platinum, and the platinum group metals. In one or more aspects, the MOC comprises one or more metals selected from the group

consisting of a Group 8 transition metal, a Group 10 transition metal, a Group 11 transition metal or combinations thereof. In one or more aspects, the MOC comprises gold (Au), palladium (Pd), platinum (Pt), iron (Fe) or combinations thereof. Generally, the metal can have any positive oxidation state available to the metal atom. In an aspect, the transition metal has an oxidation state of from +2 to +6; additionally or alternatively, from +2 to +4; additionally or alternatively, from +2 to +3 additionally or alternatively +2, +3, +4, +5, or +6. In one or more aspects, the metal is gold which can assume oxidation states ranging from -3 to +5.

[0050] In or more aspects, the MOC has one or more metals present in an amount ranging from about 0.1 weight percent (wt.%) to about 20 wt.% based on the total weight of the MOC additionally or alternatively from about 0.5 wt.% to about 15 wt.%; additionally or alternatively from about 1 wt.% to about 10 wt.%; additionally or alternatively from about 1 wt.% to about 5 wt.%; additionally or alternatively About 0.1 wt.%, about 0.25 wt.%, about 0.5 wt.%, about 0.75 wt.%, about 1 wt.%, about 1.25 wt.%, about 1.5 wt.%, about 1.75 wt.%, about 2 wt.%, about 2.25 wt.%, about 2.5 wt.%, about 2.75 wt.%, about 3 wt.%, about 3.25 wt.%, about 3.5 wt.%, about 3.75 wt.%, about 4 wt.%, about 4.25 wt.%, about 4.5 wt.%, about 4.75 wt.%, about 5 wt.%, about 5.25 wt.%, about 5.5 wt.%, about 5.75 wt.%, about 6 wt.%, about 6.25 wt.%, about 6.5 wt.%, about 6.75 wt.%, about 7 wt.%, about 7.25 wt.%, about 7.5 wt.%, about 7.75 wt.%, about 8 wt.%, about 8.25 wt.%, about 8.5 wt.%, about 8.75 wt.%, about 9 wt.%, about 9.25 wt.%, about 9.5 wt.%, about 9.75 wt.%, about 10 wt.%, about 10.25 wt.%, about 10.5 wt.%, about 10.75 wt.%, about 11 wt.%, about 11.25 wt.%, about 11.5 wt.%, about 11.75 wt.%, about 12 wt.%, about 12.25 wt.%, about 12.5 wt.%, about 12.75 wt.%, about 13 wt.%, about 13.25 wt.%, about 13.5 wt.%, about 13.75 wt.%, about 14 wt.%, about 14.25 wt.%, about 14.5 wt.%, about 14.75 wt.%, about 15 wt.%, about 15.25 wt.%, about 15.5 wt.%, about 15.75 wt.%, about 16 wt.%, about 16.25 wt.%, about 16.5 wt.%, about 16.75 wt.%, about 17 wt.%, about 17.25 wt.%, about 17.5 wt.%, about 17.75 wt.%, about 18 wt.%, about 18.25 wt.%, about 18.5 wt.%, about 18.75 wt.%, about 19 wt.%, about 19.25 wt.%, about 19.5 wt.%, about 19.75 wt.%, or about 20 wt.%.

[0051] Suitable active metal phases that may be a component of the MOC are monometallic or multimetallic combinations of copper (Cu), silver (Ag), gold (Au), nickel (Ni), palladium (Pd), platinum (Pt), iridium (Ir) or combinations thereof. In one or more aspects, the MOC comprises dopants comprising early 3d, 4d, and 5d transition metals, or heavy post transition metals such as tin (Sn), antimony (Sb), bismuth (Bi) or

combinations thereof. The introduction of dopants can alter the electronic density around the MOC active sites, resulting in increased catalytic selectivity and activity by facilitating the formation of reaction intermediates. In some aspects, the MOC comprise salkali metal modulators such as potassium (K).

[0052] In an aspect, the MOC comprises Au on a carbon support. Additionally or alternatively Au on a mesoporous support; additionally or alternatively nanoparticle gold on a carbon mesoporous support. In an aspect, the MOC comprises gold nanoparticles anchored onto a carbon support. Anchoring the gold nanoparticles more firmly to the support or by forming stable bimetallic structures, the resistance to corrosive environments is improved.

[0053] Herein, the term "active phase refers to the metal or combination of metals that form catalytic sites on the support material. The active phases can be deposited onto the support from commercially available salt precursors using incipient wetness impregnation, bulk adsorption impregnation, or deposition precipitation. The salts can then be converted to the active phase via Liquid Phase Reduction (LPR) with a formate salt at less than about 100 °C or via Gas Phase Reduction (GPR) at temperatures ranging from about 200 °C to about 500 °C. In the case of gold, calcination in air at temperatures greater than about 150 °C can also be performed.

[0054] In one or more aspects, loading of the active phase on the support is at an amount of less than about 2 wt.%. In an aspect, the loading is less than about 0.5 wt.% and the radial distribution of the active phase across the support is anisotropic. Herein "radial distribution of the active phase across the support is anisotropic" refers to how the one or metals are distributed across the support is not uniform in all directions. For example, the active phase of the MOC (i.e., catalytic metallic species) may be concentrated or differently distributed in certain directions relative to others. In an aspect, the active phase is substantially concentrated in a less than about 500 μm annulus near the surface of the extrudate support in a "core-shell" configuration. Taken together, the MOC may convert aldehyde functionalities to carboxylic acids with productivities greater than about 0.1 mol acid g^{-1}active metal h^{-1} at selectivities greater than about 80% and conversions greater than about 90% with steady state metal leaching of less than about 100 ppb based on the total weight of the MOC. In one or more aspects, the MOC may catalyze the conversion of glycolaldehyde to a product comprising glycolic acid at one or more of the following reaction parameters: temperatures ranging from about 40 °C to about 120 °C; additionally or alternatively from

about 45 °C to about 100 °C; additionally or alternatively from about 60 °C to about 80 °C and at pressures ranging from about 10 bar to about 100 bar; additionally or alternatively from about 15 bar to about 75 bar; additionally or alternatively from about 20 bar to about 60 bar .

[0055] During the oxidation of an aldehyde to a carboxylic acid in water, the pH can drop substantially to values under 2, or in some cases to a pH of about 1 via a "base-free" oxidation. In an aspect, an alkaline hydroxide (NaOH, KOH, $Ca(OH)_2$, etc.) is titrated into the reaction media to control the pH. Activity and selectivity for aldehyde oxidation to carboxylic acids are generally maximized in the pH range of from about 7 to about 12; additionally or alternatively from about 8 to about 12; additionally or alternatively from about 10 to about 12.

[0056] In some other aspects, a method of the present disclosure comprises a State 1 wherein glycolaldehyde is produced as previously described herein. In such aspects, Stage 2 of the present disclosure comprises oxidation of the glycolaldehyde in the absence of a MOC. In such aspects, the glycolaldehyde may be oxidized to form glycolic acid using another oxidase catalyst system of the type described previously herein. The oxidase catalyst system may be the same as the oxidase catalyst system used in the formation of the glycolaldehyde intermediate. In the alternative, the another oxidase catalyst system differs from the oxidase catalyst system used in the formation of the glycolaldehyde. In any aspect, the method further comprises contacting the glycolaldehyde with the another oxidase catalyst system under conditions suitable for the formation of a product comprising glycolic acid. For example, the oxidase system may comprise a GAO and the another oxidase catalyst system comprises an aldehyde oxidase (ALOD). The reaction is depicted schematically in Figure 2.

[0057] As will be understood by one of ordinary skill in the art, the reaction of ethylene glycol with an oxidase catalyst system will generate a small amount of glyoxal as a byproduct. In aspects where the Stage 1 reaction product is employed as the reactant in Stage 2, the glyoxal may be oxidized by the metal catalyst to oxalic acid. In an aspect, the Stage 2 reaction product is further processed to remove oxalic acid using any suitable methodology.

[0058] In one or more aspects, a method of the present disclosure can be carried out in a reactor system 100 of type depicted in Figure 3. With reference to Figure 3, a method of the present disclosure comprises a reactor system for the production of glycolic acid 100. In an aspect, reaction components may be disposed in containers in fluid

communication with downstream units. In an aspect, the reaction components,110 and 120, are introduced to an enzyme oxidation reactor (EOR) 140 which is also in fluid communication with air and water streams, 115 and 117 respectively. In one or more aspects, the oxidation of ethylene glycol is carried out in the EOR 140 under the conditions previously described. The reacted mixture may exit the EOR 140 and be subjected to any number of purification procedures (e.g., nanofiltration 150). In some aspects, a means of ensuring proper water flow is present in the reactor system (e.g., break tank 160). The purified product mixture may be conveyed to a metal oxidation reactor (MOR) 170 having an MOC disposed therein. In one or more aspects, the MOR 170 is in fluid communication with a caustic solution 130 that may be used to raise the pH of the reaction media in the MOR 170. In such aspects, the purified product mixture serves a reactant that is subjected to further oxidation in the MOR under conditions of the type previously disclosed herein to produce a final product mixture. The final product mixture exiting the MOR 170 may contain some amount of glycolic acid, designated glycolic product mixture (GPM) that may be conveyed to a suitable vessel. The GPM may be suitable for use in one or more applications without further processing. In some aspects, the GPM may be further processed in order to increase the concentration and/or purity of the glycolic acid present in the GPM.

[0059] Figure 3 is a depiction of reactor system 100 utilizing 3 reactors, however systems utilizing a single continuous stirred tank slurry reactor (CSTR) or greater than 3 fixed bed reactors of various sizes with or without interstage cooling and interstage caustic injection are also contemplated. Similarly, the enzymatic reactor and a sparged bubble column (as depicted) or an air lift column or a falling film high pressure oxidation. Although Figure 3 is at process flow diagram level of detail, not all process interconnections are shown such as spillbacks, block and bleeds, recycle lines, control valves, cooling/heating elements, pumps, intermediate tankage, antifoam, etc.

[0060] Disclosed herein are methods and compositions suitable for the formation of glycolic acid from ethylene glycol. Any method of the present disclosure may further comprise the recovery of at least a portion of any intermediate and/or product disclosed herein. Further, any method disclosed herein may further comprise the use of purification, concentration or isolation techniques to purify, concentrate and/or isolate a reaction product of the methods employed herein.

[0061] In an aspect, the compositions, methods and processes disclosed herein result in the production of glycolic acid having a purity of from about 70% to about 99%;

additionally or alternatively from about 80% to about 95%; additionally or alternatively greater than about 95%. As disclosed herein, the use of purified or partially pure enzymes as catalysts eliminates the presence of the majority of byproducts observed in conventional glycolic acid synthesis thereby facilitating separation and purification of the product and leading to lower production costs. Further, the methods disclosed herein result in the formation of high purity glycolic acid using an *in-vitro* system. The methods disclosed herein utilizing a biocatalyst (e.g., oxidase catalyst system) and a metal catalyst (e.g., MOC) or a combination of biocatalysts to generate glycolic acid reduces the need for hazardous chemicals used in the conventional methods of generating glycolic acid such as formaldehyde and carbon monoxide. Further environmental benefits of the present disclosure may be realized by deriving the substrate (e.g., ethylene glycol) from renewable resources rather than fossil fuels, thereby providing a pathway to a greener process than the conventional methods.

[0062] In aspects, one or more of molecules of the present disclosure are biobased molecules characterized by equal to or greater than about 70% of the carbon atoms in the molecule originating from a renewable resource; additionally or alternatively equal to or greater than about 75%; additionally or alternatively equal to or greater than about 80%; additionally or alternatively equal to or greater than about 85%; additionally or alternatively equal to or greater than about 90%. Herein a renewable resource refers to a natural resource which will replenish to replace the portion depleted by usage and consumption, either through natural reproduction or other recurring processes in a finite amount of time on a human time scale.

ADDITIONAL DISCLOSURE

[0063] The following are additional nonlimiting exemplary aspects of the presently disclosed subject matter

[0064] A first aspect which is a method for the production of glycolic acid comprising contacting ethylene glycol with an oxidase catalyst system under conditions suitable for the formation of glycolaldehyde; contacting glycolaldehyde with one or more metal oxidation catalysts under conditions suitable for the formation of glycolic acid; and recovering at least a portion of the glycolic acid.

[0065] A second aspect which is the method of the first aspect wherein the oxidase catalyst system comprises (i) one or more oxidase enzymes, and (ii) one or more cofactors.

[0066] A third aspect which is the method of the second aspect wherein the one or more oxidase enzymes comprises a copper radical oxidase, a galactose oxidase, an alcohol oxidase, a glycerol oxidase, an unspecific peroxygenase, mutants thereof, fragments thereof or combinations thereof.

[0067] A fourth aspect which is the method of any of the second through third aspects wherein the one or more oxidase enzymes has any of SEQ ID NO:1 through SEQ ID NO:11.

[0068] A fifth aspect which is the method of any of the second through fourth aspects wherein the one of more oxidase enzymes comprise a mutated galactose oxidase.

[0069] A sixth aspect which is the method of any of the second through fifth aspects wherein the cofactor comprises a single electron oxidizer.

[0070] A seventh aspect which is the method of the sixth aspect wherein the single electron oxidizer comprises 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 and combinations thereof.

[0071] An eighth aspect which is the method of any of the second through fifth aspects wherein the cofactor comprises a small molecule activator.

[0072] A ninth aspect which is the method of the eighth aspect wherein the small molecule activator is selected from the group consisting of 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.

[0073] A tenth aspect which is the method of any of the second through fifth aspects wherein the cofactor is selected from the group consisting of thiamine pyrophosphate, NAD+, NADP+, pyridoxal phosphate, methyl cobalamin, cobalamine, biotin, Coenzyme

A, tetrahydrofolic acid, menaquinone, ascorbic acid, flavin mononucleotide, flavin adenine dinucleotide, and Coenzyme F420.

[0074] An eleventh aspect which is the method of any of the first through tenth aspects wherein the oxidase catalyst system further comprises a catalase.

[0075] A twelfth aspect which is the method of any of the first through eleventh aspects wherein the metal oxidation catalyst comprises (a) a metal and (b) a support material.

[0076] A thirteenth aspect which is the method of the twelfth aspect wherein the support comprises ceramic, metal oxides, glass, titania, silica, alumina, zirconia, ceria, ceramic, carbon or combinations thereof.

[0077] A fourteenth aspect which is the method if any of the twelfth through thirteenth aspects wherein the support material has a surface area of from about 100 m^2/g to about 1000 m^2/g.

[0078] A fifteenth aspect which is the method of any of the twelfth through fourteenth aspects wherein the support material has a pore volume of from about 0.10 cc/g to about 0.4 cc/g.

[0079] A sixteenth aspect which is the method of any of the twelfth through fifteenth aspects wherein the support material has less than about 20% micropores.

[0080] A seventeenth aspect which is the method of any of the twelfth through sixteenth aspects wherein the metal has an oxidation state of from +2 to +6.

[0081] An eighteenth aspect which is the method of any of the twelfth through seventeenth aspects wherein the metal comprises gold (Au), palladium (Pd), platinum (Pt), iron (Fe) or combinations thereof.

[0082] A nineteenth aspect which is the method of any of the first through eighteenth aspects wherein the glycolic acid has a purity of from about 70% to about 99%.

[0083] A twentieth aspect which is a method for the production of glycolic acid comprising contacting ethylene glycol with an oxidase catalyst system comprising galactose oxidase under conditions suitable for the formation of glycolaldehyde; contacting glycolaldehyde with an oxidation catalyst comprising gold on a carbon support under conditions suitable for the formation of glycolic acid; and recovering at least a portion of the glycolic acid.

[0084] A twenty-first aspect which is the method of the twentieth aspect wherein the carbon support has a surface area of from about 100 m^2/g to about 1000 m^2/g.

[0085] A twenty-second aspect which is the method of any of the twentieth through twenty-first aspects wherein the carbon support has a pore volume of from about 0.10 cc/g to about 0.4 cc/g.

[0086] A twenty-third aspect which is the method of any of the twentieth through twenty-second aspects wherein the carbon support has less than about 20% micropores.

[0087] A twenty-fourth aspect which is the method of any of the twentieth through twenty-third aspects wherein the glycolic acid has a purity of from about 70% to about 99%.

EXAMPLES

[0088] The presently disclosed subject matter having been generally described, the following examples are given as particular aspects of the subject matter and to demonstrate the practice and advantages thereof. It is understood that the examples are given by way of illustration and are not intended to limit the specification or the claims in any manner.

Example Protocol for Enzyme Testing

[0089] Colorimetric Microtiter Plate Enzymes (wildtype and mutants) will be screened using a microtiter plate-base colorimetric assay that monitors the production of hydrogen peroxide. Organisms that may be screened include *Achatina achatina, Achatina fulica, Arion ater, Aspergillus ochraceus, Aspergillus ochraceus AIU 031, Aspergillus nidulans, Aspergillus terreus, Aspergillus terreus MTCC 6324, BasidiomycotaBasidiomycota B191039, Byssochlamys spectabilis, Byssochlamys spectabilis RI01, Paecilomyces variotii, Candida boidinii, Candida methanolica, Candida koshuensis, Candida olivarium, Candida ooitensis, Candida queretana, Candida silvicola, Hanensula alcoolica, Kloeckera boidinii, Torulopsis enokii, Candida cariosilignicola, Candida guilliermondii, Pichia guilliermondii, Yamadazyma guilliermondii, Endomyces guilliermondii, Candida methanolovescens, Ogatea minuta, Candida methanosorbosa, Candida methanosorbosa M-2003, Candida sithepensis, Candida sonorensis, Torulopsis sonorensis, Candida sp. (in: Saccharomycetales), Candida sp. (in: Saccharomycetales) 25-A, Candida succiphila, Candida tropicalis, Comamonas sp., Comamonas sp. UVS, Gloeophyllum trabeum, Hansenula polymorpha, Ogataea polymorpha, Pichia angusta, Hanensula angusta Ogataea angusta, Ogataea angusta DL-1, Ogataea angusta NCYC 495, Helix aspersa, Kuraishia capsulata, Lachnellula arida, Lachnellula cervina, Lachnellula occidentalis,*

Lachnellula subtilissima, Lachnellula suecica, Lachnellula willkommii, Methylococcus capsulatus, Methylophilus methylotrophus, Ochrobactrum sp., Ochrobactrum sp. AIU 033, Ogataea glucozyma, Ogataea henricii, Ogataea methanolica, Pichia pinus, Ogataea minuta, Ogataea naganishii, Ogataea philodendri, Ogataea pignalia, Ogataea pini, Ogataea siamensis, Ogataea trehalophila, Ogataea wickerhamii, Passalora fulva, Penicillium chrysogenum, Penicillium purpurascens, Penicillium purpurascens AIU 063, Phanerochaete chrysosporium, Phanerochaete chrysosporium DSMZ 1547, Phanerochaete chrysosporium K-3, Phlebiopsis gigantea, Pichia pastoris, Komagataella pastoris, Komagataella phaffii, Komagataella pseudopastoris, Endomyces pastoris, Petasospora pastorisZygosaccharomyces pastoris, Zygowillia pastoris, Zymopichia pastoris, Komagataella pastoris GS115, Komagataella pastoris IFP 206, Komagataella pastoris X33, Pichia putida, Polyporus obtusus, Poria contigua, Radulodon casearius, Thodotorula toruloides, Thermoascus aurantiacus, Thermoascus aurantiacus NBRC 31693, Trametes cinnabarina, and *Cavia porcellus* Screening for the production of hydroperoxide may carried out using any suitable methodology. For example, the reagents o-dianisidine and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) may be used in conjunction with horseradish peroxidase to elicit a color change in the presence of hydrogen peroxide. Enzymes or combination of enzymes are diluted to total stock concentration of 1 mg/mL and then diluted further into a roughly 200 µL volume containing glucose substrate, the reporter molecule, buffer, and horseradish peroxidase. The dilution factor is chosen such that the change in color falls within the range of 0.01-0.06 absorbance units per minute as measured with a plate-based spectrophotometer. This rate of change can be used along with the dilution factor and extinction coefficient of the reporter molecule to calculate the specific activity of the enzyme(s) or plotted to select mutants with high activity for further characterization.

Parr bomb Scale Testing

[0090] Once suitable enzymes have been identified through the screening process, a pressurized Parr bomb system will be used to mimic the reactor conditions at scale in order to assess the efficacy of converting glucose to glucaric acid using the candidate enzymes. In a final volume of 50 mL, purified enzymes at 1-0.001% w/v will be combined with 20% w/v glucose and a buffer (typically phosphate buffer) at an initial pH of 4-7. Catalase may be added at a 1:1 to 1:20 oxidative enzyme to catalase ratio to prevent accumulation of hydrogen peroxide. The mixture will be loaded into the Parr bomb

containing a stir bar. To improve mass transfer of oxygen into the solution, the vessel will be sparged with oxygen two times, then pressurized to 100 atm. The reactor will be held at constant temperature, typically 20 °C but within the range of 10-60 °C and the mixture allowed to react until the reaction is complete. During the reaction, the vessel may be depressurized to adjust the pH and obtain samples to assess conversion and product profile. Testing methods may include monitoring pH decrease as acids are produced, a colorimetric o-dianisidine assay to monitor formation of hydrogen peroxide, and HPLC for the detection of glycolaldehyde, glycolic acid, glyoxal, oxalic acid and the like.

[0091] 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, 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, 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.

[0092] Accordingly, the scope of protection is not limited by the description set out above but is only limited by the claims which follow, 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 present invention. 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 method for the production of glycolic acid, the method comprising:

contacting ethylene glycol with an oxidase catalyst system under conditions suitable for the formation of glycolaldehyde;

contacting glycolaldehyde with one or more metal oxidation catalysts under conditions suitable for the formation of glycolic acid; and

recovering at least a portion of the glycolic acid.

2. The method of claim 1, wherein the oxidase catalyst system comprises (i) one or more oxidase enzymes, and (ii) one or more cofactors.

3. The method of claim 2, wherein the one or more oxidase enzymes comprises a copper radical oxidase, a galactose oxidase, an alcohol oxidase, a glycerol oxidase, an unspecific peroxygenase, mutants thereof, fragments thereof or combinations thereof.

4. The method of claim 2, wherein the one or more oxidase enzymes has any of SEQ ID NO:1 through SEQ ID NO:11.

5. The method of claim 2, wherein the one of more oxidase enzymes comprise a mutated galactose oxidase.

6. The method of claim 2, wherein the cofactor comprises a single electron oxidizer.

7. The method of claim 6, wherein the single electron oxidizer comprises 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 and combinations thereof.

8. The method of claim 6, wherein the cofactor comprises a small molecule activator.

9. The method of claim 8, wherein the small molecule activator is selected from the group consisting of 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.

10. The method of claim 6, wherein the cofactor is selected from the group consisting of thiamine pyrophosphate, NAD+, NADP+, pyridoxal phosphate, methyl cobalamin, cobalamine, biotin, Coenzyme A, tetrahydrofolic acid, menaquinone, ascorbic acid, flavin mononucleotide, flavin adenine dinucleotide, and Coenzyme F420.

11. The method of claim 1, wherein the oxidase catalyst system further comprises a catalase.

12. The method of claim 1, wherein the metal oxidation catalyst comprises (a) a metal and (b) a support material.

13. The method of claim 12, wherein the support comprises ceramic, metal oxides, glass, titania, silica, alumina, zirconia, ceria, ceramic, carbon or combinations thereof.

14. The method of claim 12, wherein the support material has a surface area of from about 100 m^2/g to about 1000 m^2/g.

15. The method of claim 12, wherein the support material has a pore volume of from about 0.10 cc/g to about 0.4 cc/g.

16. The method of claim 12, wherein the support material has less than about 20% micropores.

17. The method of claim 12, wherein the metal has an oxidation state of from +2 to +6.

18. The method of claim 12, wherein the metal comprises gold (Au), palladium (Pd), platinum (Pt), iron (Fe) or combinations thereof.

19. The method of claim 1, wherein the glycolic acid has a purity of from about 70% to about 99%.

20. A method for the production of glycolic acid, the method comprising:

contacting ethylene glycol with an oxidase catalyst system comprising galactose oxidase under conditions suitable for the formation of glycolaldehyde;

contacting glycolaldehyde with an oxidation catalyst comprising gold on a carbon support under conditions suitable for the formation of glycolic acid; and

recovering at least a portion of the glycolic acid.

21. The method of claim 20, wherein the carbon support has a surface area of from about 100 m^2/g to about 1000 m^2/g.

22. The method of claim 20, wherein the carbon support has a pore volume of from about 0.10 cc/g to about 0.4 cc/g.

23. The method of claim 20 wherein the carbon support has less than about 20% micropores.

24. The method of claim 20, wherein the glycolic acid has a purity of from about 70% to about 99%.

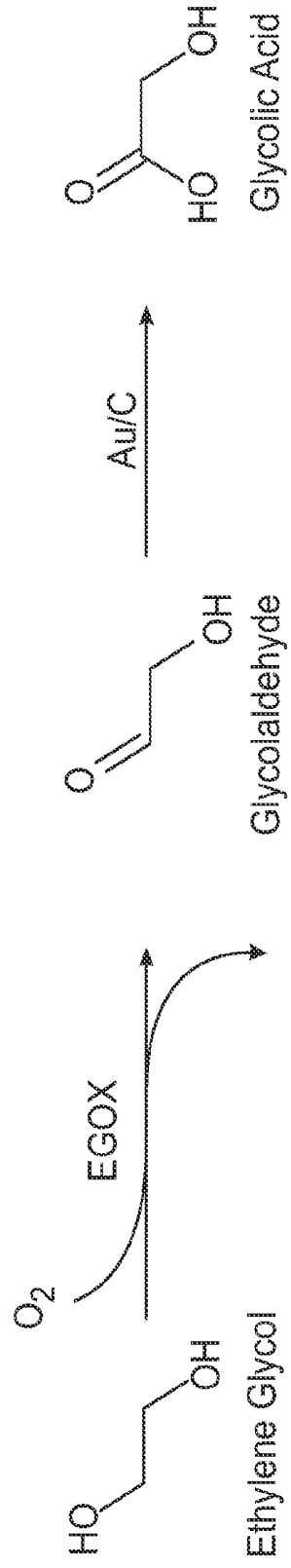
HO
OH
Ethylene Glycol
O_2
EGOX
O
OH
Glycolaldehyde
Au/C
O
HO
OH
Glycolic Acid
H_2O_2
H_2O_2
Catalase
$2H_2O + O_2$

FIG. 1

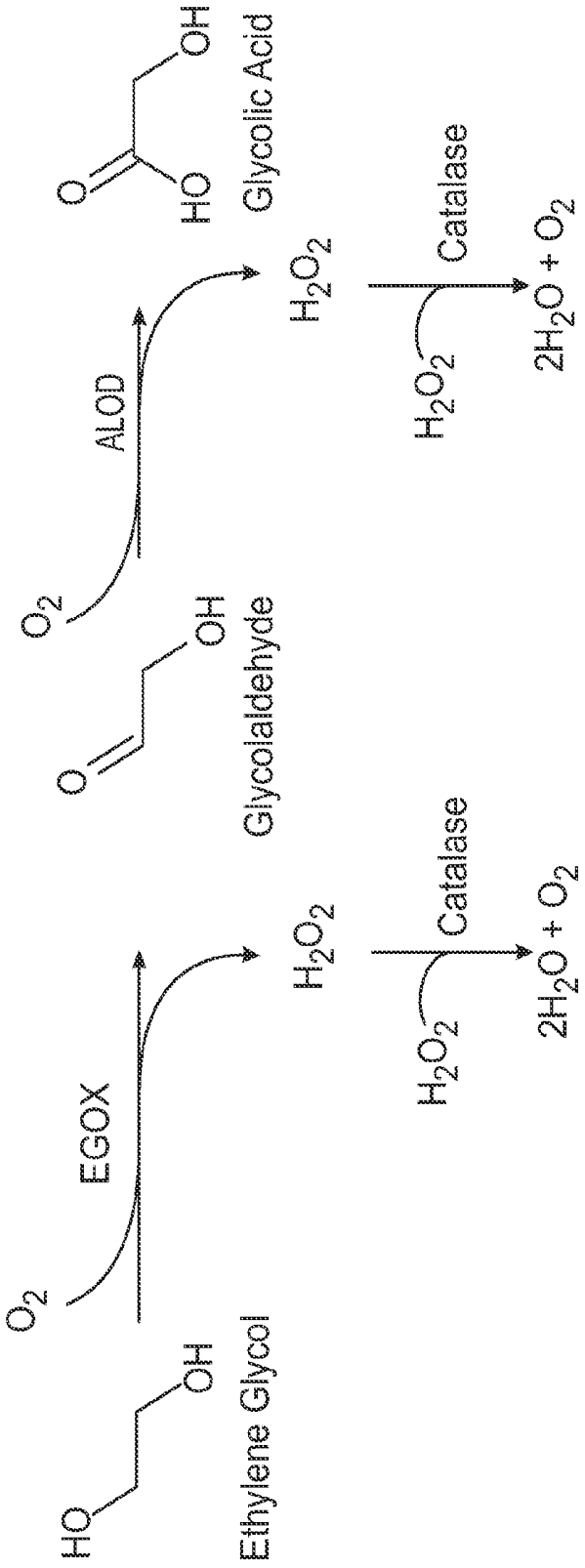
HO
OH
Ethylene Glycol
EGOX
O_2
H_2O_2
Catalase
H_2O_2
$2H_2O + O_2$
O
OH
Glycolaldehyde
ALOD
O_2
H_2O_2
Catalase
H_2O_2
$2H_2O + O_2$
O
HO
OH
Glycolic Acid

FIG. 2

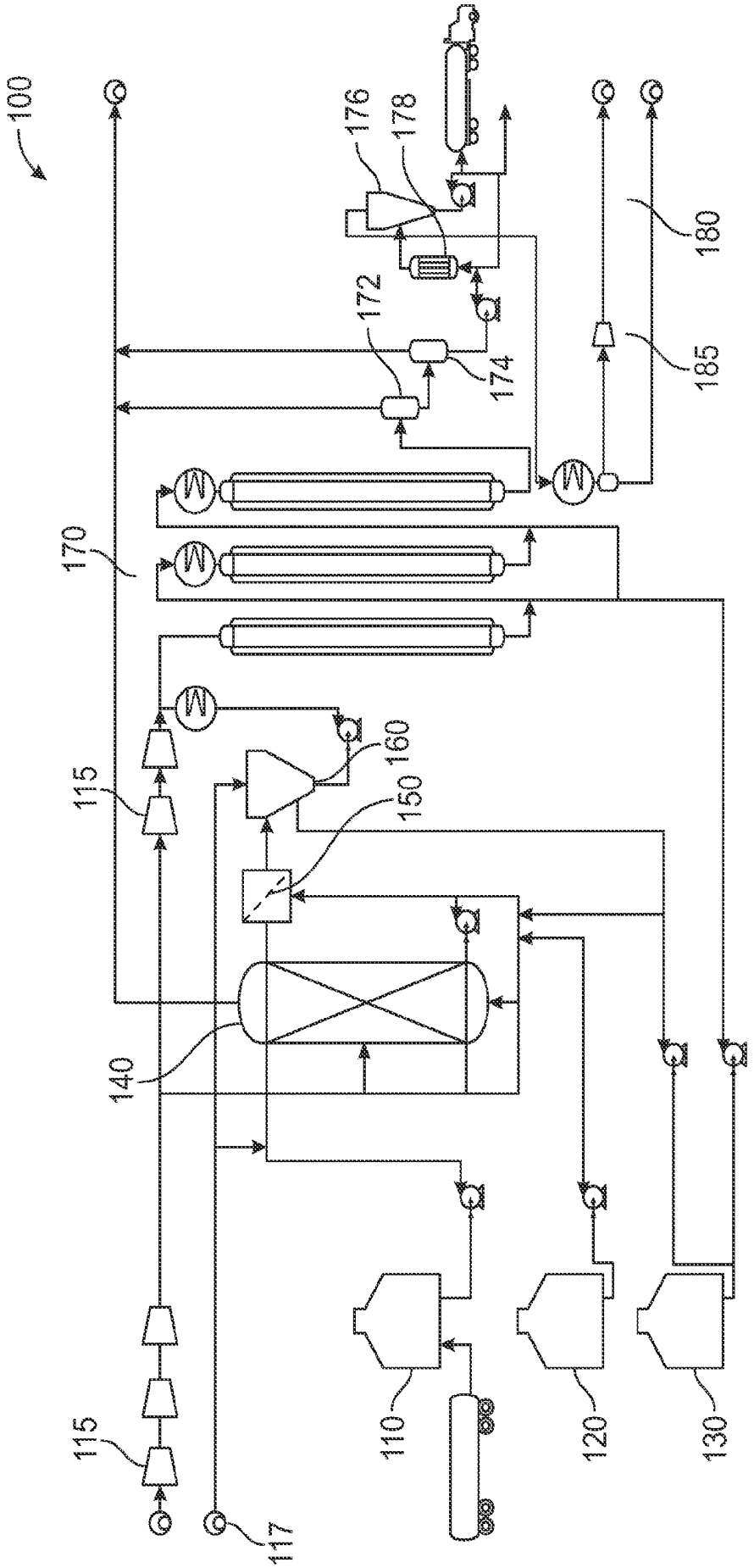
100
176
178
180
172
174
185
170
115
160
150
140
115
117
110
120
130

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2025/032974

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12P 7/02*** (2023.01); ***C12P 7/24*** (2023.01); ***C12P 7/40*** (2023.01); *B01J 23/66* (2023.01)

CPC: ***C12P 7/02; C12P 7/24; C12P 7/40;*** *B01J 23/66; C12Y 101/03009*

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.
A	WO 2023/004432 A2 (SOLUGEN, INC.) 26 January 2023 (26.01.2023) para [00125], [00127], [00137]-[00138], [00152], [00170]-[00171], [00228], Fig. 16	1-7, 12-19
A	US 2021/0252485 A1 (Shibasaki) 19 August 2021 (19.08.2021) abstract	1-7, 12-19
A	Puetz et al. "Biocatalytic Oxidation of Alcohols" Catalysts. 20 August 2020, vol 10, pg. 1-30 pg. 11, Scheme 8, para 1	1-7, 12-19
A	Zhang et al. "Oxidation of ethylene glycol to glycolaldehyde using a highly selective alcohol dehydrogenase from Gluconobacter oxydans" Journal of Molecular Catalysis B: Enzymatic. 29 December 2014, vol 112, pg. 69-75 entire document	1-7, 12-19
A	Zhao et al. "Recent advances of ethylene glycol oxidation reaction: catalytic mechanism, catalyst design and applications" Journal of Materials Chemistry A. 11 December 2024, vol 13, pg. 3236-3272 entire document	1-7, 12-19

☐ 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
03 October 2025 (03.10.2025)	**08 October 2025 (08.10.2025)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450** **UNITED STATES OF AMERICA**	**HARRY KIM**
Facsimile No. **571-273-8300**	Telephone No. **PCT Help Desk: 571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2025/032974

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.

Group I+: Claims 1-24, directed to a method for the production of glycolic acid, the method comprising: contacting ethylene glycol with an oxidase catalyst system under conditions suitable for the formation of glycolaldehyde; contacting glycolaldehyde with one or more metal oxidation catalysts under conditions suitable for the formation of glycolic acid; and recovering at least a portion of the glycolic acid. The method of claim 1 will be searched to the extent that it encompasses the first species of claim 1, represented by a method for the production of glycolic acid, the method comprising: contacting ethylene glycol with an oxidase catalyst system, which is the first oxidase catalyst system of claim 2, wherein the oxidase catalyst system comprises (i) one oxidase enzyme, which is the first oxidase enzyme of claim 4, SEQ ID NO: 1, and (ii) one cofactor, which is the first cofactor of claim 6, a single electron oxidizer, which is the first single electron oxidizer of claim 7, a laccase; under conditions suitable for the formation of glycolaldehyde; contacting glycolaldehyde with one or more metal oxidation catalysts, which is the first metal oxidation catalyst of claim 12, a metal and a solid material; wherein the metal is the first metal of claim 18, gold; and the support is the first support of claim 13, ceramic; under conditions suitable for the formation of glycolic acid; and recovering at least a portion of the glycolic acid (wherein optional groups are omitted). It is believed that claims 1-7 and 12-19 read on this first named invention, and thus these claims will be searched without fee. This first named invention has been selected 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 compounds of claim 1, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. 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. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be a method for the production of glycolic acid, the method comprising: contacting ethylene glycol with an oxidase catalyst system, which is the first oxidase catalyst system of claim 2, wherein the oxidase catalyst system comprises (i) one oxidase enzyme, which is the first oxidase enzyme of claim 4, SEQ ID NO: 1, and (ii) one cofactor, which is the first cofactor of claim 6, a single electron oxidizer, which is the first single electron oxidizer of claim 7, a laccase; under conditions suitable for the formation of glycolaldehyde; contacting glycolaldehyde with one or more metal oxidation catalysts, which is the first metal oxidation catalyst of claim 12, a metal and a solid material; wherein the metal is the first metal of claim 18, gold; and the support is carbon; under conditions suitable for the formation of glycolic acid; and recovering at least a portion of the glycolic acid (wherein optional groups are omitted) (i.e., claims 1-7 and 12-24).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2025/032974

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.: **1-7 and 12-19**

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.