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(54) **MICROORGANISM MEDIATED LIQUID FUELS**

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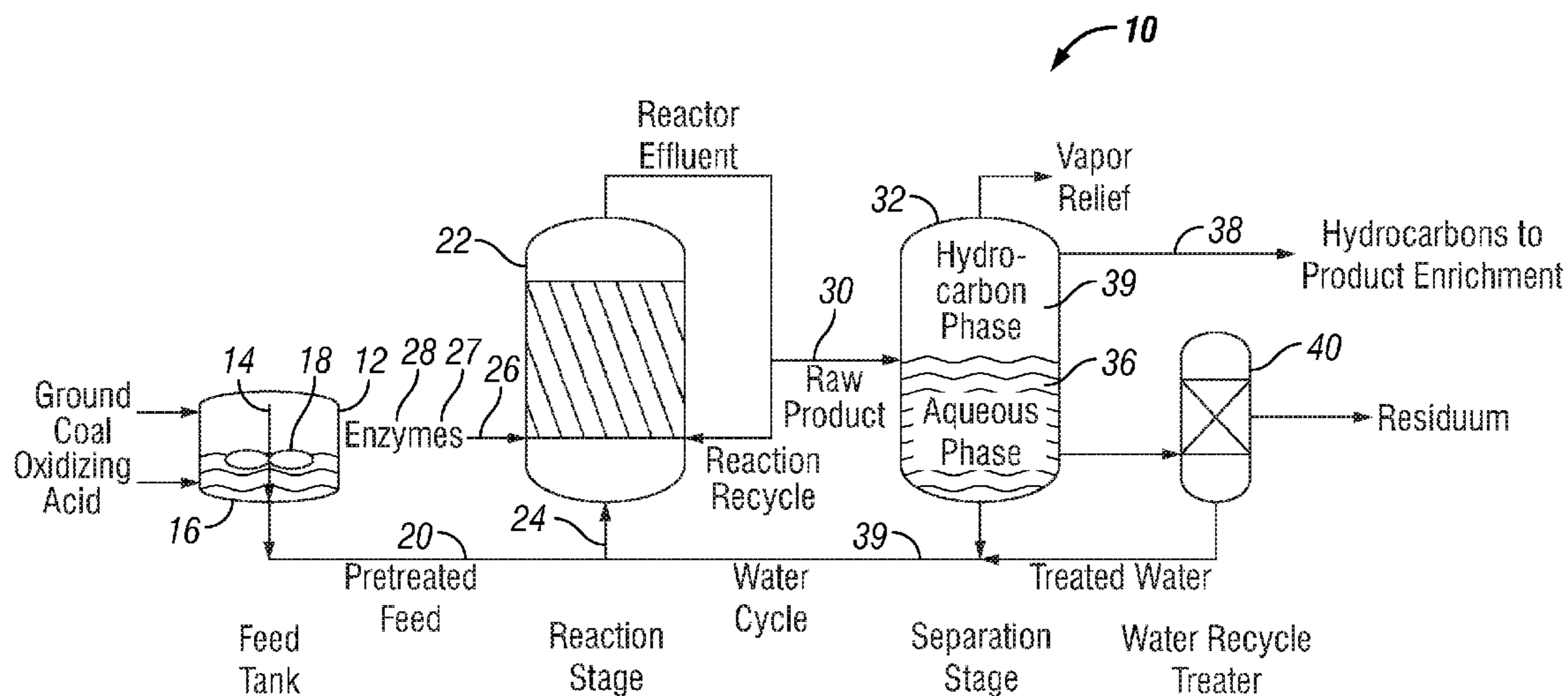
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(57) **ABSTRACT**

A method for producing a liquid fuel from a hydrocarbon source. In one embodiment, the method comprises disintegrating a hydrocarbon source, treating the disintegrated hydrocarbon source, solubilizing the disintegrated hydrocarbon source, admixing a biochemical liquor, wherein the biochemical liquor comprises at least one conversion enzyme to form liquid hydrocarbons, separating liquid hydrocarbons, and enriching the liquid hydrocarbons to form a liquid hydrocarbon product. Further, the method comprises producing a liquid fuel in-situ. In certain embodiments the method comprises modified enzymes for producing the liquid fuels.

Related U.S. Application Data

(60) Provisional application No. 61/141,552, filed on Dec. 30, 2008, provisional application No. 61/146,816, filed on Jan. 23, 2009.



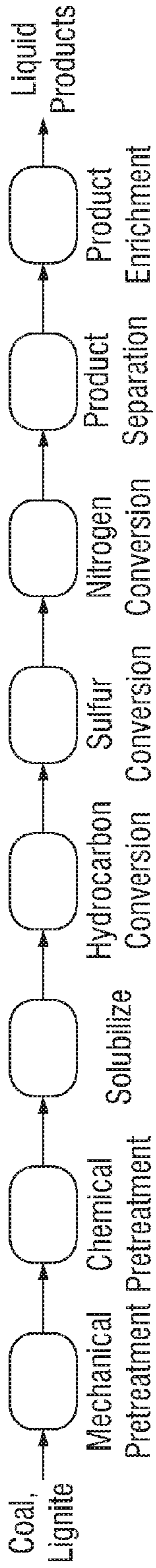


FIG. 1

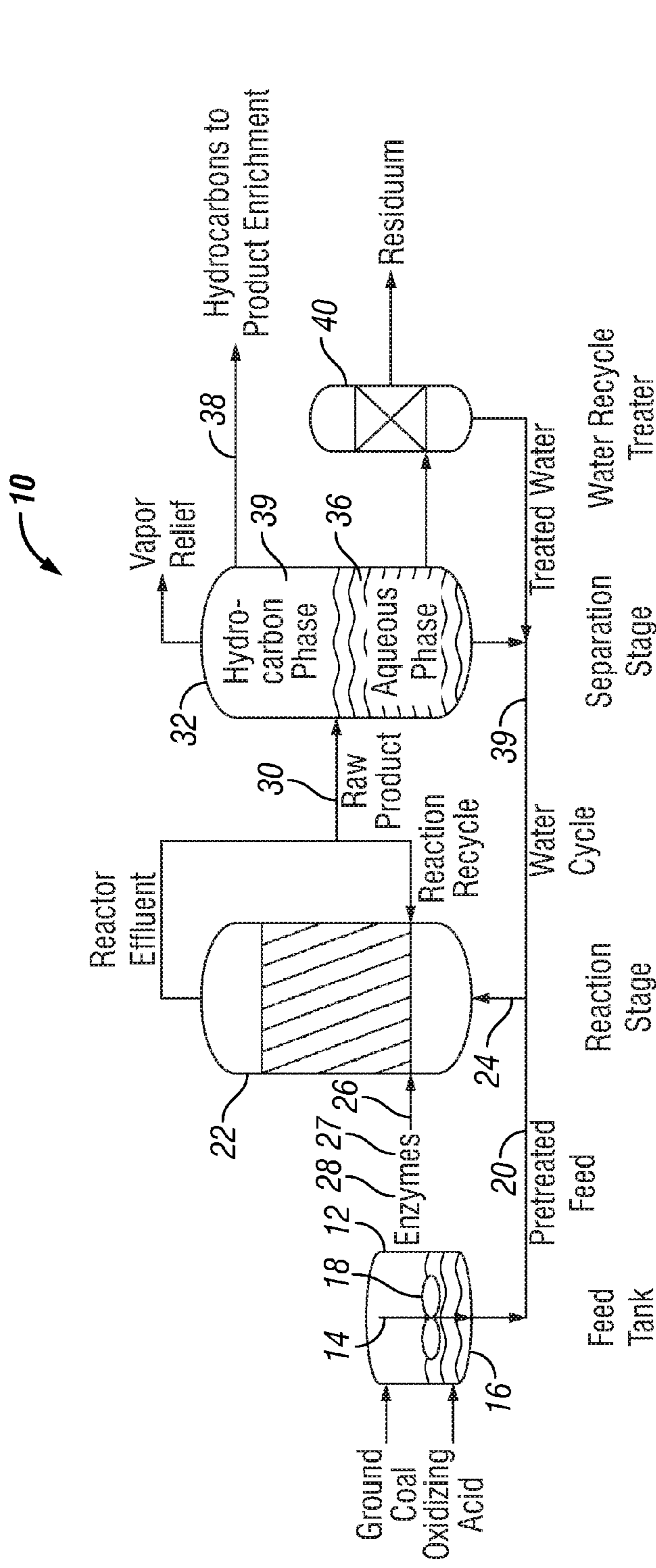


FIG. 2

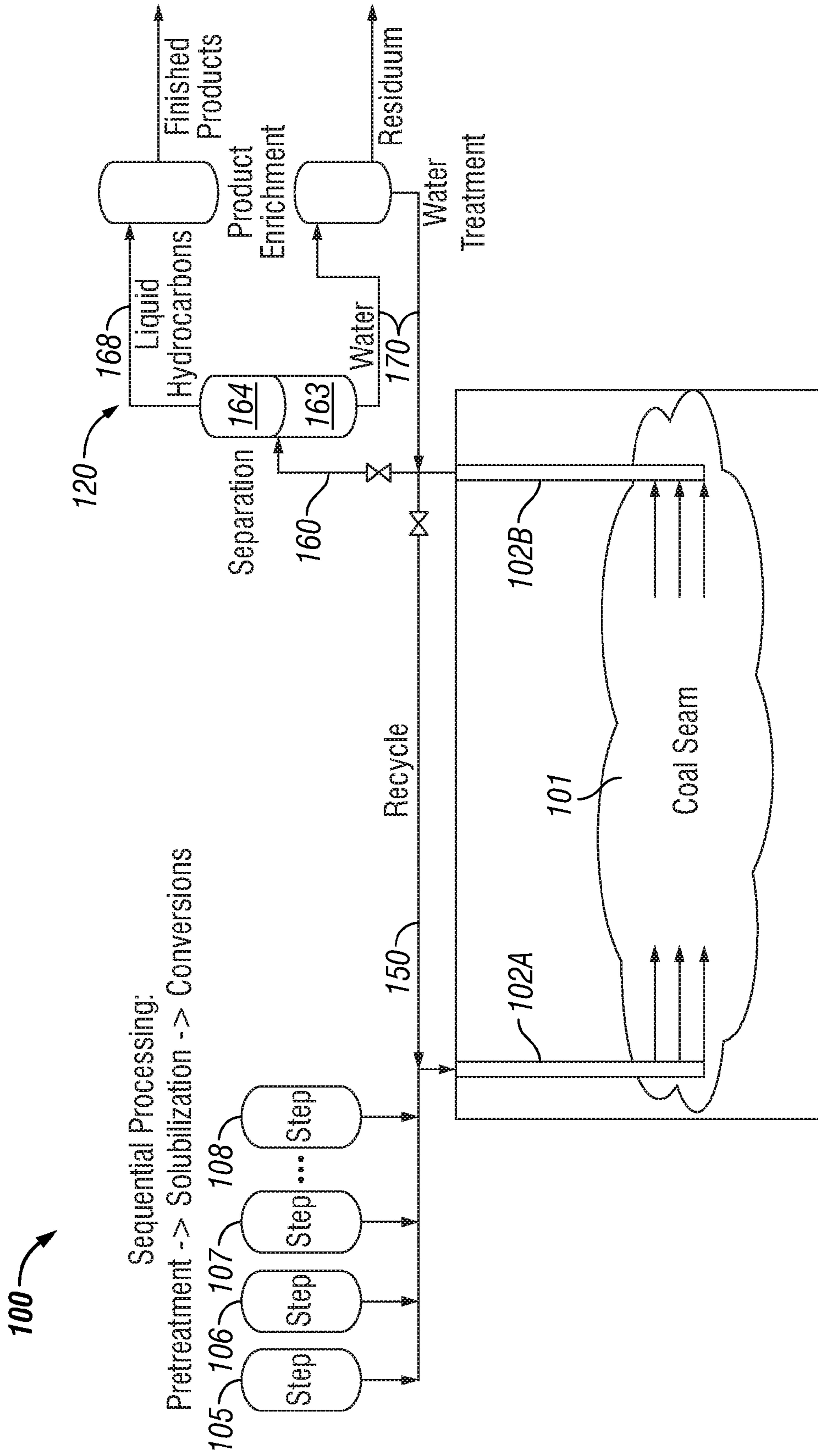


FIG. 3

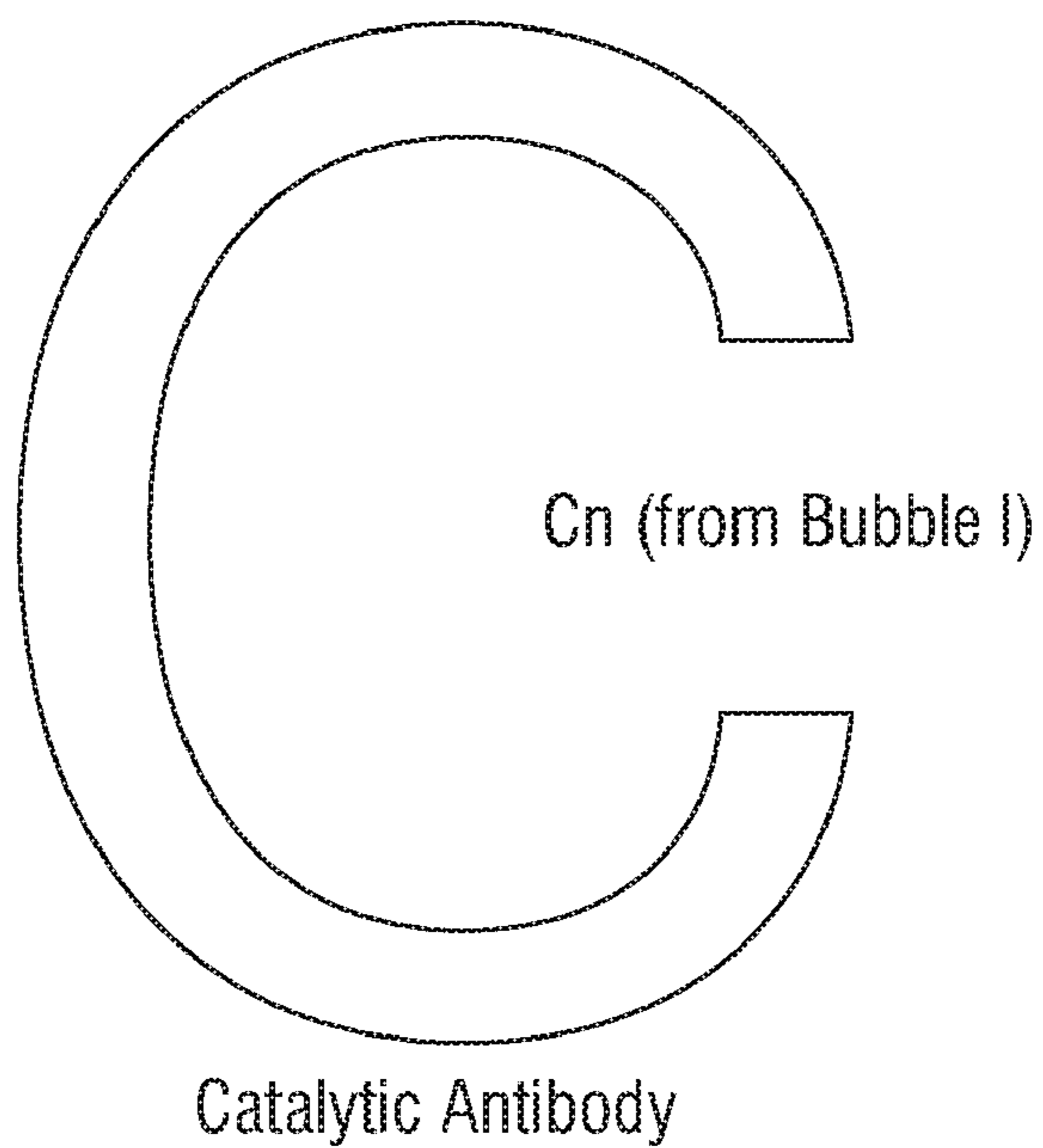


FIG. 4

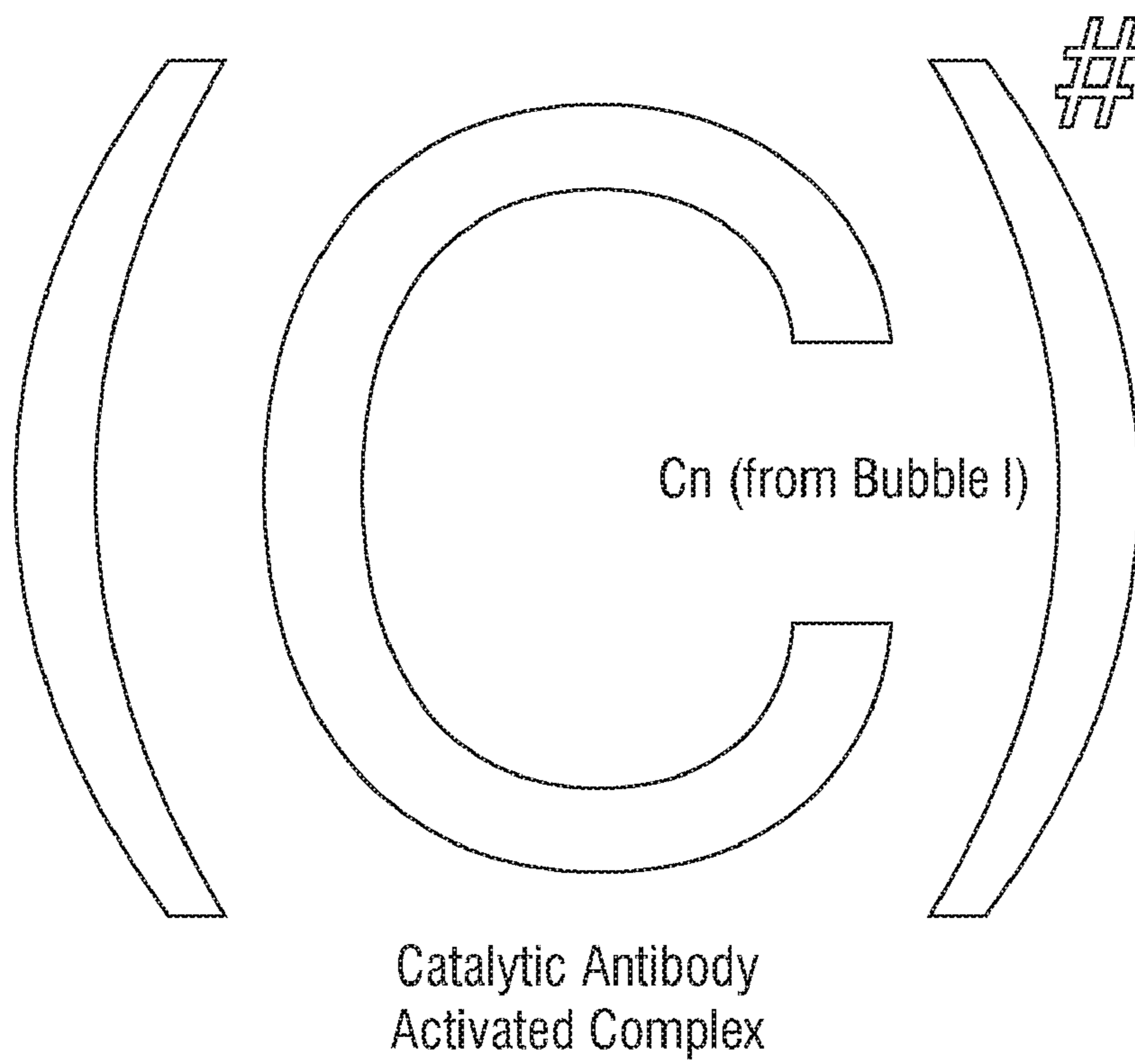
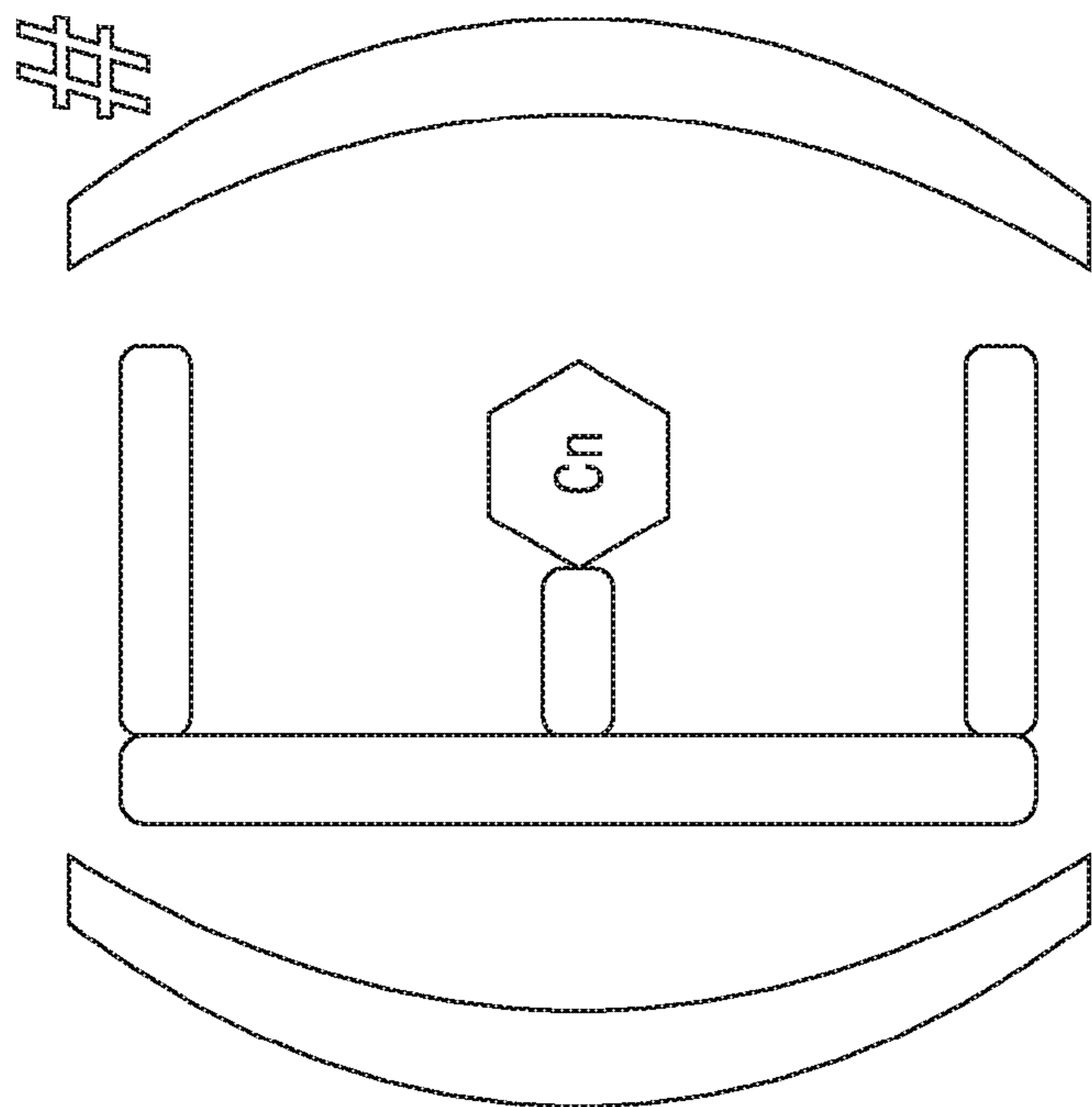


FIG. 5



Mother Nature Only Enzyme - Wild

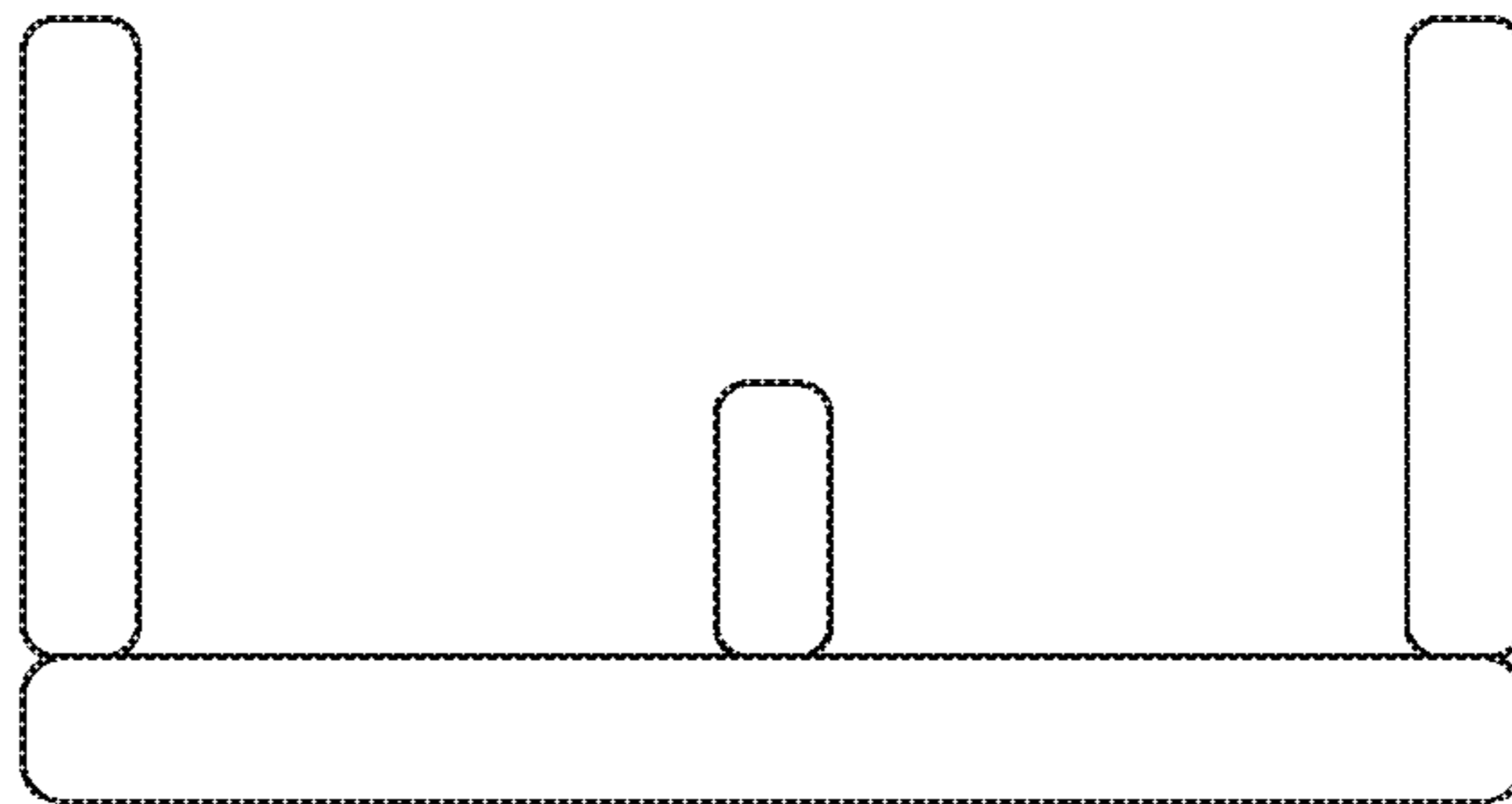
Type MNO - WT

Cn (From Bubble I)

(E-C_n)#

Activated Complex

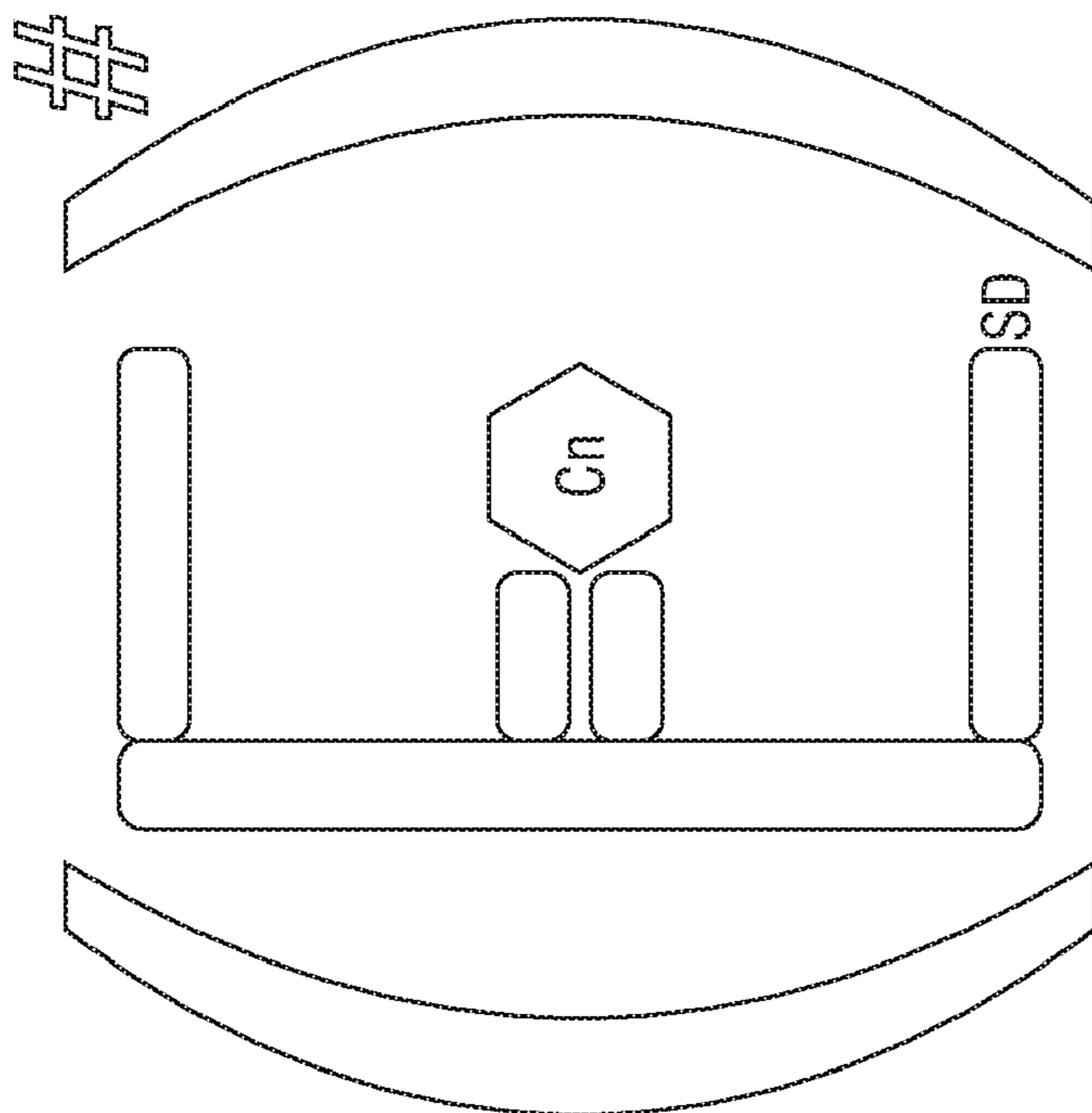
FIG. 7



Mother Nature Only Enzyme - Wild

Type MNO - WT

FIG. 6



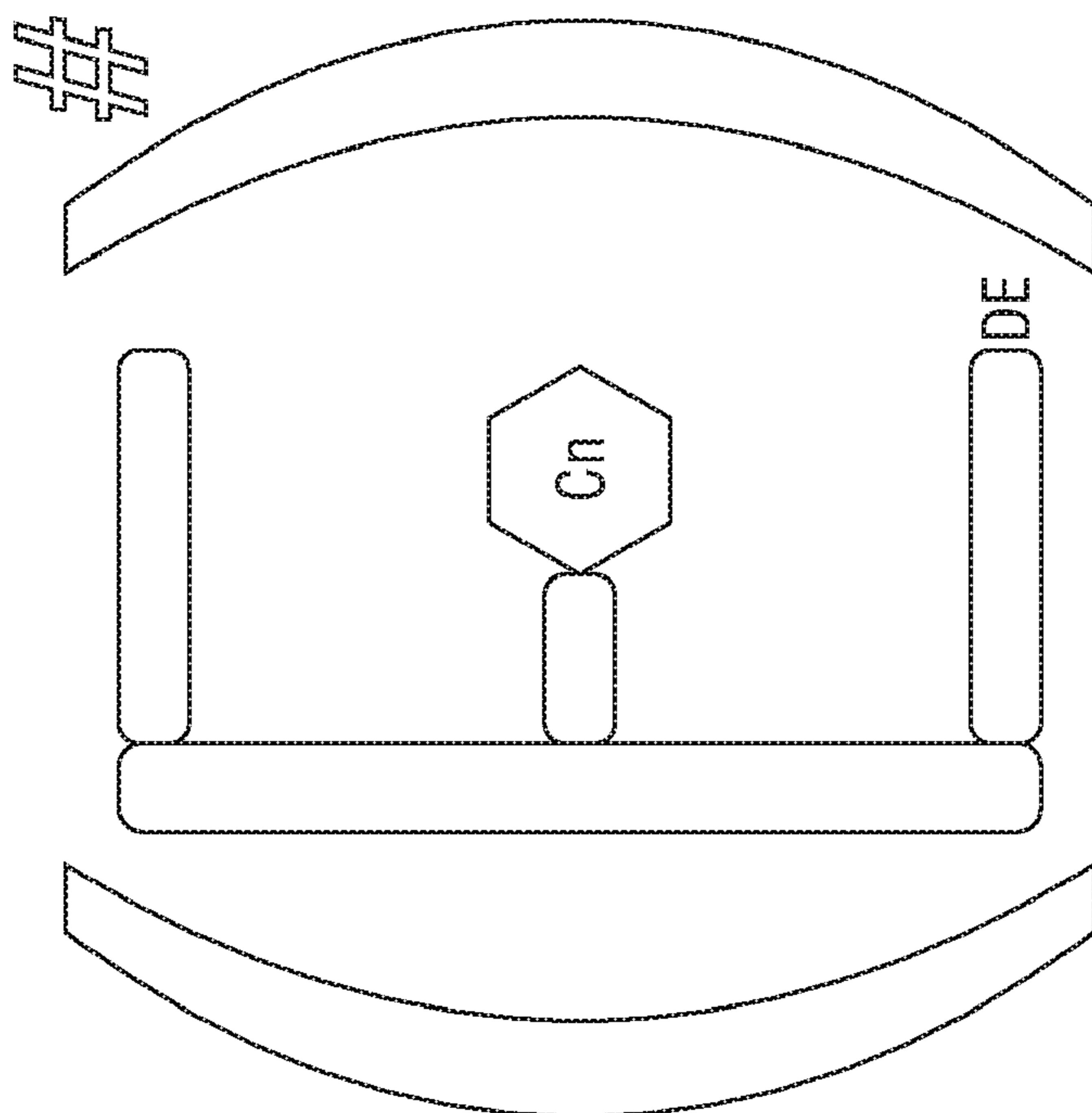
Site Directed Mutagenesis

Cn (From Bubble I)

(ESD-C_n)#

Activated Complex

FIG. 9



Mother Nature Only Enzyme -
Directed Evolution MNO - DE

Cn (From Bubble I)

(EDE-C_n)#

Activated Complex

FIG. 8

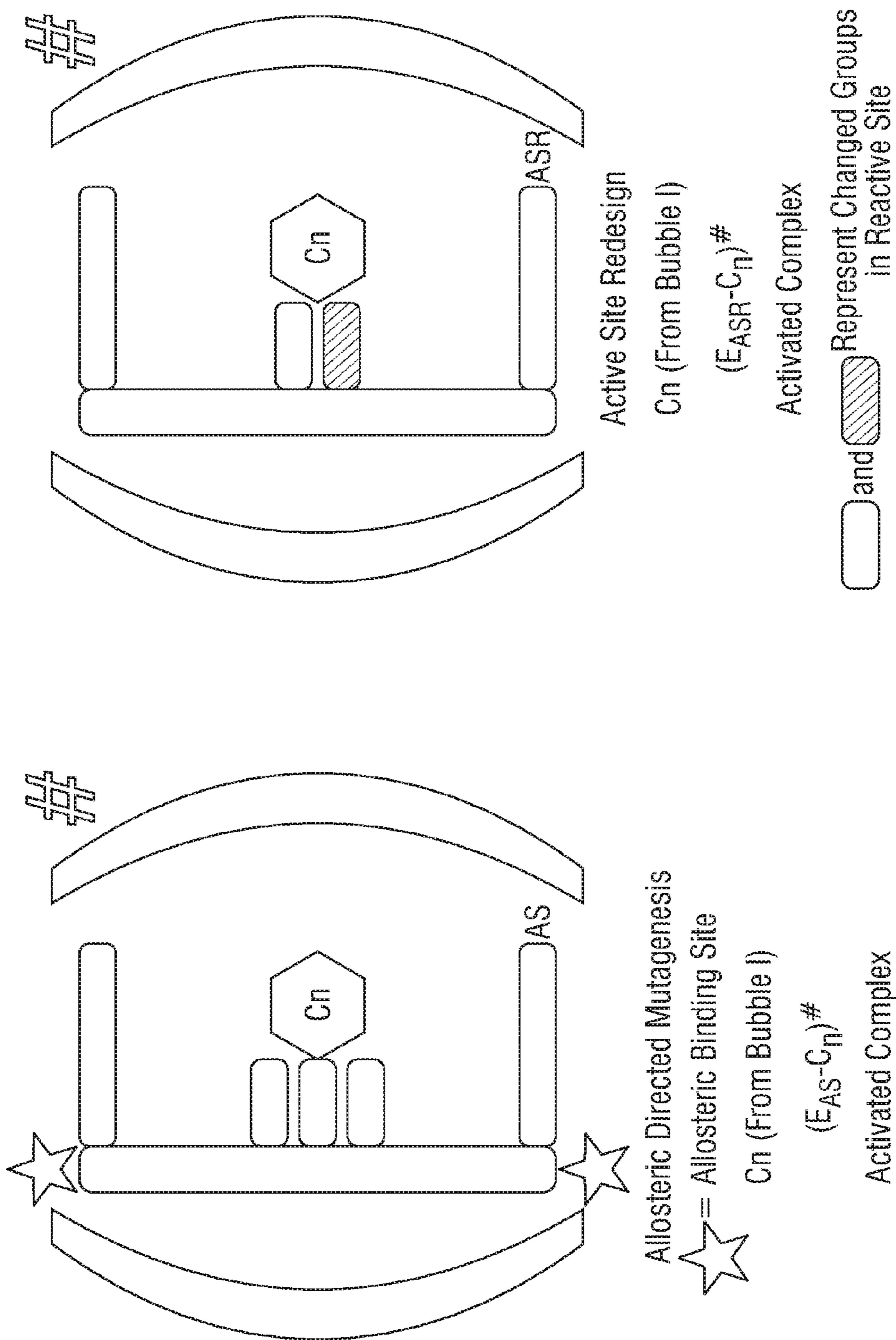


FIG. 11

FIG. 10

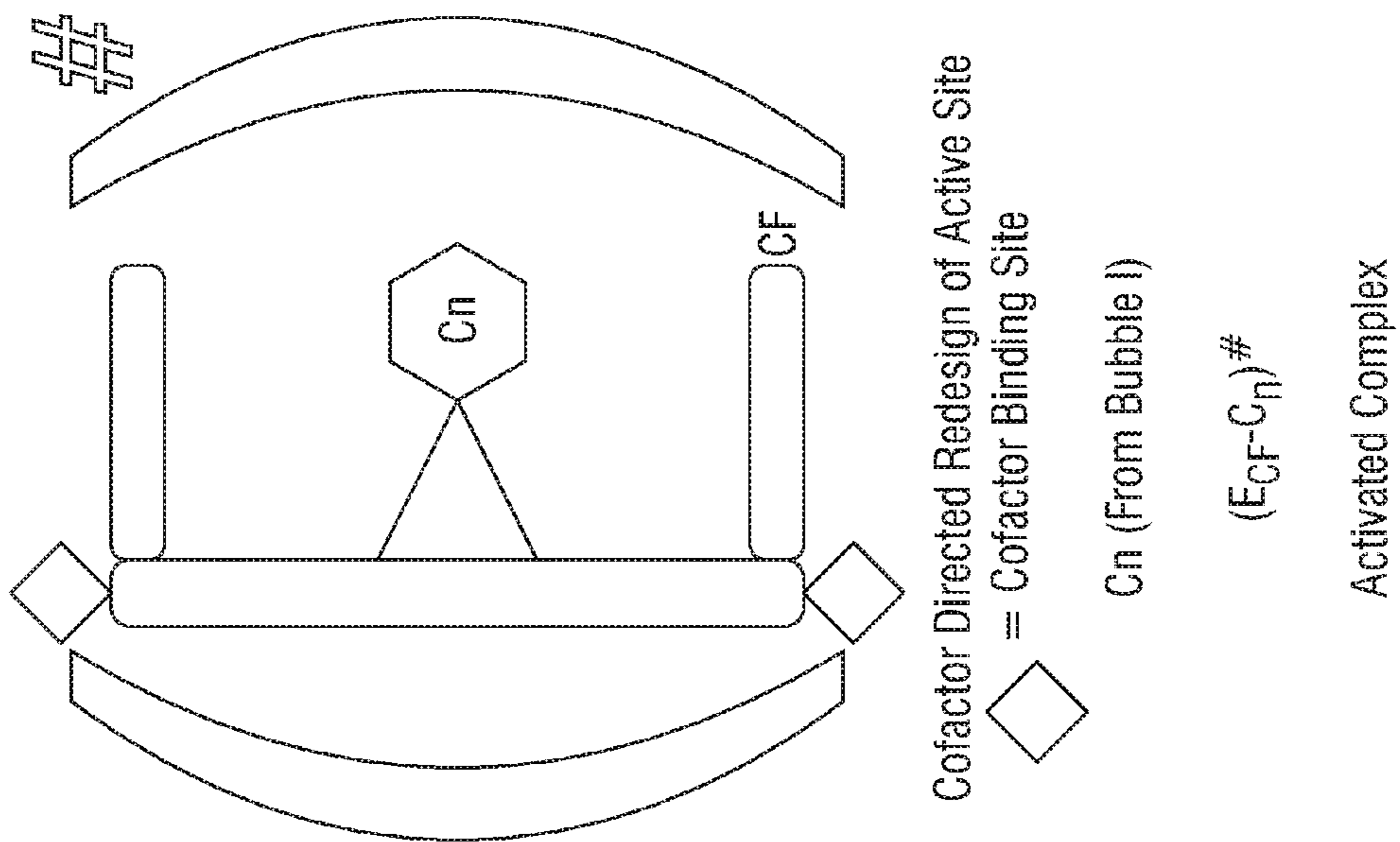


FIG. 13

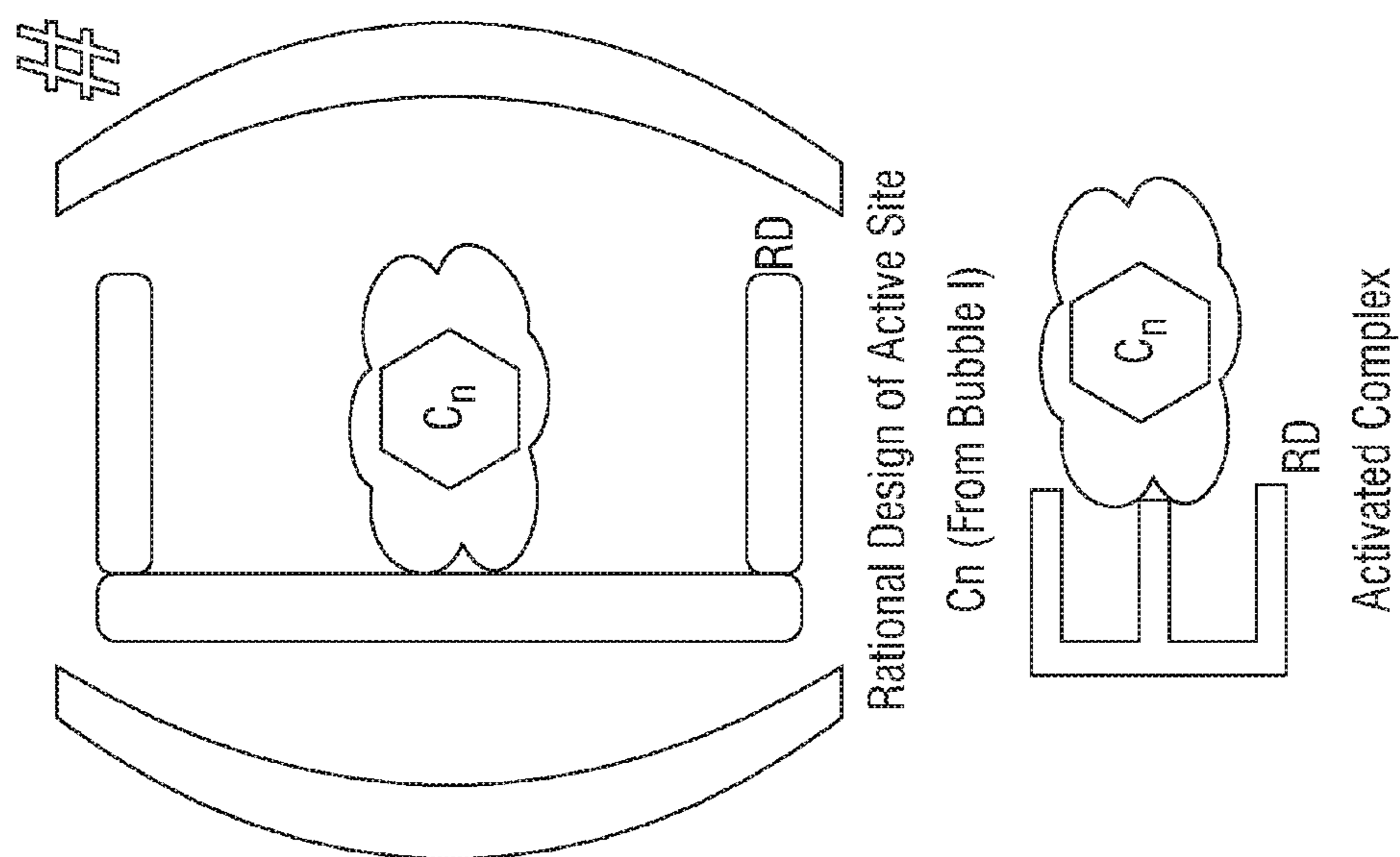


FIG. 12

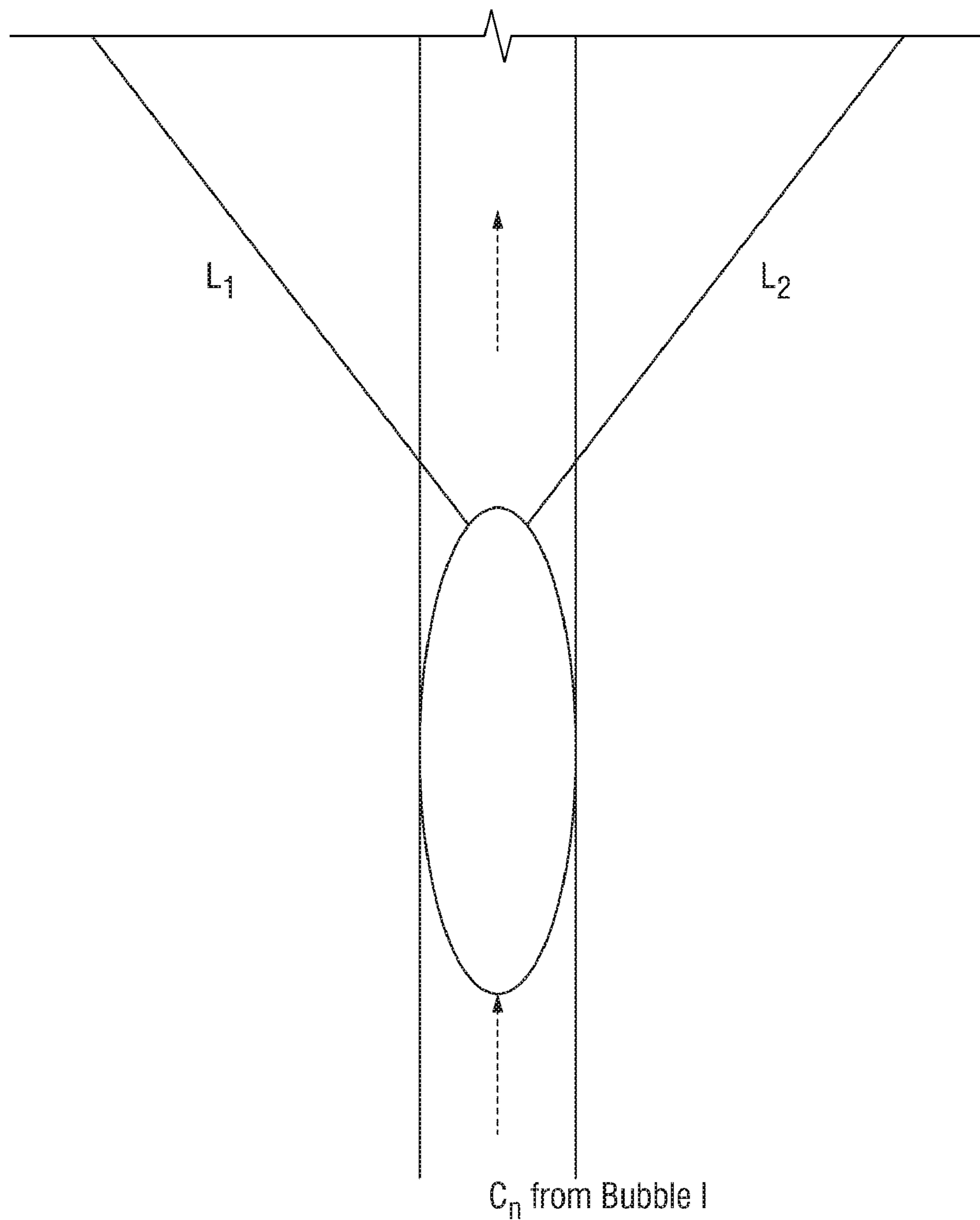
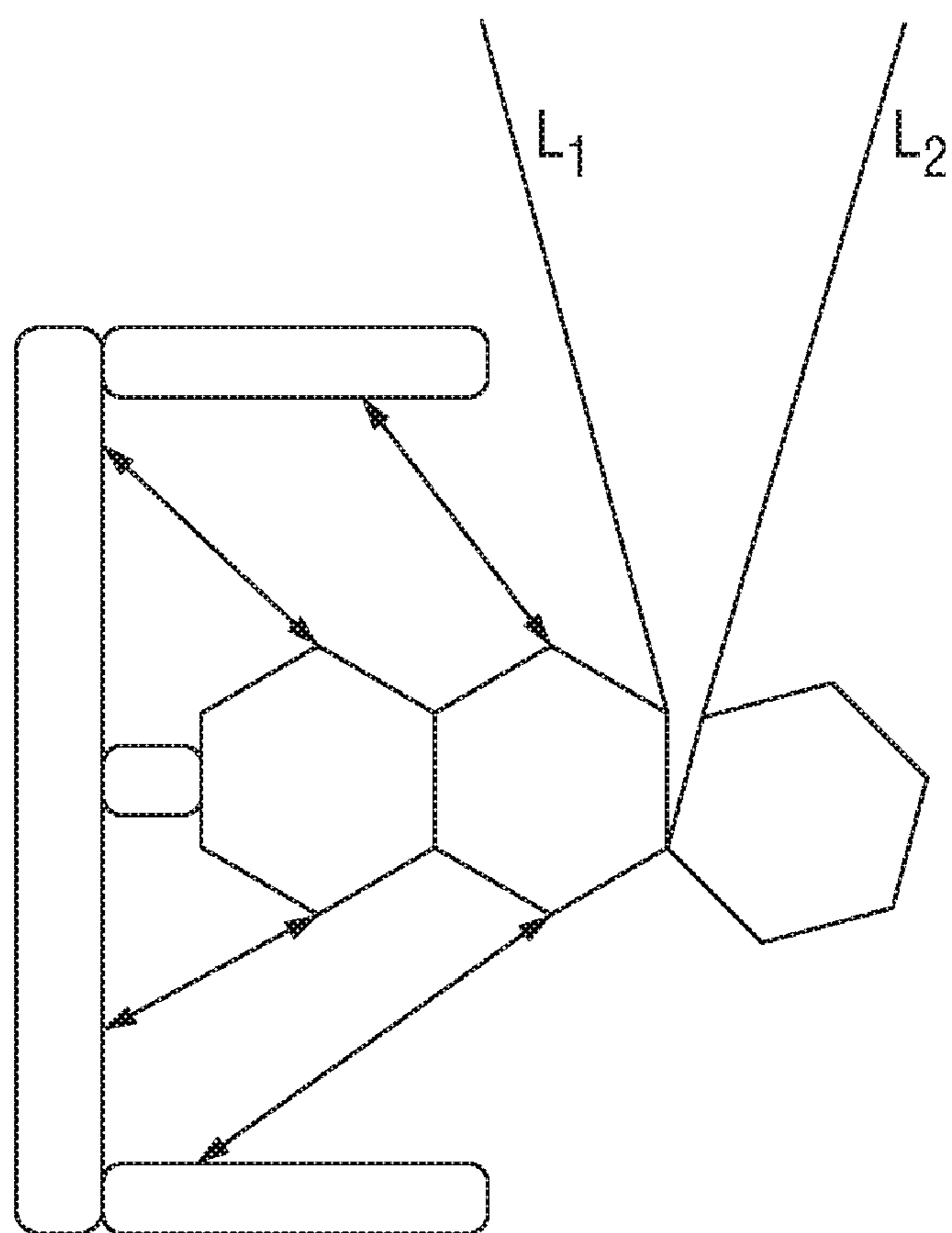


FIG. 14





 Represent Non Covalent Bonds Between
 Enzymes/ Enzyme-Like Structures and C_n Reactants
 from Bubble I. C_n Reactants Depicted as Ring
 Structures for Illustration Purposes Only

FIG. 15

MICROORGANISM MEDIATED LIQUID FUELS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application No. 61/141,552 filed Dec. 30, 2008 and U.S. Provisional Patent Application No. 61/146,816 filed Jan. 23, 2009 the disclosures of which are hereby incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

BACKGROUND

[0003] 1. Field of the Invention

[0004] This invention relates to producing liquid fuels, specifically to in-situ or ex-situ coal to liquid conversion.

[0005] 2. Background of the Invention

[0006] Coals can also be converted into liquid fuels like gasoline or diesel by several different processes. In a developing commercial process, the coal converted into a gas first, and then into a liquid, by using the Fischer-Tropsch (FT) process. In the FT process, an indirect route, coal is first gasified to make syngas, a purified mixture of CO and H₂ gas. Next, FT catalysts are used to convert the syngas into light hydrocarbons, like ethane, which are further processed into refinable liquid fuels. In addition to creating the fuels, syngas can also be converted into methanol, which can be used as a fuel, or a fuel additive.

[0007] Alternatively, the coal may be converted directly to liquid fuels via hydrogenation processes. For example, the Bergius process, in which coal is liquefied by mixing it with hydrogen gas and heating the system. Several other direct liquefaction processes have been developed, such as the Solvent Refined Coal (SRC) processes, which has spawned several pilot plant facilities. Additionally, dried, pulverized coal mixed with roughly 1 wt % molybdenum catalysts may be hydrogenated by use of high temperature and pressure synthesis gas produced. However, the syngas must be produced in a separate gasifier.

[0008] However, these coal to liquid fuel processes involve the mining of the coal from the ground. As is well documented, coal mining is a hazardous process, and many mines are forced into closure prior to the removal of all usable products. Further, those mines that are operated safely leave behind large columns of coal to support the ceiling and coal residues in the mine walls. These sources of coal represent a significant amount of energy that is left abandoned by mining operations. Further, these untouched resources may be converted to liquid fuels for transportation purposes. As such, there is a need in the industry for the removal of abandoned, low quality, or residual coal from mining operations, for use in the coal to liquid production.

BRIEF SUMMARY

[0009] In one embodiment, a method for producing liquid hydrocarbon products, comprising, disintegrating a hydrocarbon source, treating the disintegrated hydrocarbon source chemically, solubilizing the disintegrated hydrocarbon source, admixing a biochemical liquor, wherein the biochemical liquor comprises at least one enzyme to form liquid

hydrocarbons, separating liquid hydrocarbons, and enriching the liquid hydrocarbons to form a liquid hydrocarbon product.

[0010] In another embodiment, a method for in-situ coal to liquid hydrocarbon conversion, comprising, locating an underground coal seam, drilling at least one well, the well in contact the underground coal seam; pressurizing the underground coal seam with steam; cycling reactants through the underground coal seam, wherein the reactants comprise at least one enzyme, to form a slurry; withdrawing a portion of the slurry; processing the slurry, wherein the liquid hydrocarbon is separated from the slurry; and returning the slurry to the coal seam for further processing.

[0011] In further embodiments, a method for using an enzyme to produce liquid fuels, comprising selecting a microorganism, the microorganism producing an enzyme; modifying a microorganism genetically, to increase the production of the enzyme; modifying the enzyme structurally, to alter the activity of the enzyme, to form a modified enzyme; collecting the modified enzyme to form a biochemical liquor comprising at least one modified enzyme; and exposing a hydrocarbon source to the biochemical liquor to form a liquid fuel precursor.

[0012] The foregoing has outlined rather broadly the features and technical advantages of the invention in order that the detailed description of the invention that follows may be better understood. The various characteristics described above, as well as other features, will be readily apparent to those skilled in the art upon reading the following detailed description of the preferred embodiments, and by referring to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] For a detailed description of the preferred embodiments of the invention, reference will now be made to the accompanying drawings if various embodiments of the invention, in which:

[0014] FIG. 1 illustrates a general flow process schematic for converting coal to liquid.

[0015] FIG. 2 illustrates one embodiment of an ex-situ process for converting coal to liquid.

[0016] FIG. 3 illustrates one embodiment of an in-situ process for converting coal to liquid.

[0017] FIG. 4 illustrates a representative diagram of a catalytic antibody.

[0018] FIG. 5 illustrates a representative diagram of an activated catalytic antibody.

[0019] FIG. 6 illustrates a representative diagram of a wild type enzyme.

[0020] FIG. 7 illustrates a representative diagram of an activated enzyme complex.

[0021] FIG. 8 illustrates a representative diagram of a directed evolution of an activated enzyme complex.

[0022] FIG. 9 illustrates a representative diagram of site directed mutagenesis of an activated enzyme complex.

[0023] FIG. 10 illustrates a representative diagram of allosteric directed mutagenesis of an activated enzyme complex.

[0024] FIG. 11 illustrates a representative diagram of an active site redesign of an activated enzyme complex.

[0025] FIG. 12 illustrates a representative diagram of an active site rational design of an activated enzyme complex.

[0026] FIG. 13 illustrates a representative diagram of a cofactor directed active site redesign of an activated enzyme complex.

[0027] FIG. 14 illustrates a schematic of photofragmentation.

[0028] FIG. 15 illustrates a schematic of laser mediated photofragmentation.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0029] A process for converting coal to liquid fuels is disclosed. Coal comprises any coal found or removed from a coal mine, seam, or pit. The coal may further comprise anthracite coals, or the coke from bituminous coal. In certain instances, the coal comprises lignite, sub-bituminous coal, other low-rank coals, and/or other hydrocarbon source, such as tar sands, without limitation. Alternatively, the coal comprises weathered, aged, leached, or degraded coal without limitation.

[0030] In certain instances, the coal is converted to liquid fuels by a two-stage process. The process comprises enzymatically-catalyzed reactions. The first stage, Stage I, comprises the pretreatment and conversion of coal into liquid products by enzymes. In embodiments, Stage I converts coal feedstocks to liquid hydrocarbons. The feedstocks comprise coal remaining in coal mines, or in-situ feedstocks. Alternatively, the feedstocks comprise coal away from the coal mine, or ex-situ feedstocks. In certain instances, the source of hydrocarbons may comprise any source, such as tar sands, not preferred for other industries.

[0031] The second stage, Stage II, comprises the enrichment of the liquid hydrocarbon products. The enrichment, or improvement, of the liquid hydrocarbon product comprises further enzymatically-catalyzed reactions. Additionally, the reactions are enzyme-mediated processing steps. Stage II further comprises the improvement of the liquid product properties for use in fuels. The Stage II enzymatically-catalyzed processes change the fuel performance. In exemplary instances, Stage II processing may alter the cetane rating of diesel, or the octane rating of gasoline.

[0032] Compared to current processing techniques, the enzymatically-mediated two-stage process requires less energy for processing. Additionally, the reaction conditions are milder, as the enzymes perform optimally in homeostatic conditions of the microorganism producer. Further, additional enzymes or microorganisms may be implemented to sequester contaminants regulated in liquid fuels. For instance, sulfur and nitrogen may be reduced or removed from the liquid fuels prior to final product distillation. The early removal of polluting contaminants makes the process adjustable to meet current and future emissions regulations. Additionally, the enzyme-mediated two-stage process is adaptable to feedstocks previously inaccessible, for instance, coal columns in a mine too dangerous for removal by retreat mining.

[0033] Microorganisms. In the disclosed process, microorganism-produced enzymes mediate the conversion. In embodiments, the microorganisms comprise bacteria, algae, or fungi. In certain instances, the microorganisms comprise heterotrophs that secrete enzymes for catalytic digestion of hydrocarbons. The microorganisms use hydrocarbons as a carbon source for life processes. The microorganisms are harvested from oil shales, oil sands, coal tar pits, or coal caves without limitation. For example, the microorganisms may be derived from those found in the La Brea Tar Pits. Further, the microorganisms may be collected from geothermal springs, mud volcanoes, sulfur cauldrons, fumaroles, geysers, mud-

pots, or the like, without limitation. The microorganisms may further comprise extremophiles, such as but not limited to hypoliths, endoliths, cryptoliths, acidophiles, alkaliphiles, thermophiles, lithoautotrophs, halophiles, or piezophiles. In exemplary embodiments, the microorganisms comprise archaeobacteria. Alternatively, suitable microorganisms include those found in the genera *Poria*, *Polyporus*, *Thiobacillus*, *Candida*, *Streptomyces*, *Pseudomonas*, *Penicillium*, or *Trichoderma*. As understood by one skilled in the art, alternative microorganisms may be identified that are suitable for application in the disclosed system, without being named specifically, that do not vary in structure and function significantly. Further, it can be envisioned that these microorganisms are envisioned as means to alter, improve, or modify the current disclosure.

[0034] In certain instances, the microorganisms are exposed to previously mined coal, coal residues, or coal residues less favorable for power production in order to harvest the enzymes. In instances, the microorganism produces the enzymes naturally. As understood by one skilled in the art, continued exposure to the substrate, such as coal, will lead to increased expression and production of the enzymes for the liquid hydrocarbon production.

[0035] Enzymes. The enzymes used in the disclosed process are obtained from microorganisms that produce these enzymes in high yields. In further embodiments, the microorganisms are genetically altered to produce the enzymes in high yields. Preferably, the enzymes are secreted extracellularly and/or release the enzymes into their environment. Alternatively, the cells are lysed and the enzymes are captured for use in coal processing. In embodiments, the enzymes are separated from the microorganisms prior to use in the processing of coal. In order to reduce exposure, release, or environmental contamination, the microorganisms are separated from the processing. No microorganisms are directly involved in any embodiments of the liquid fuel production process. Furthermore, the facilities used to grow these organisms have sufficient provisions to isolate host organisms from the natural environment.

[0036] Alternatively, the microorganisms undergo site-directed mutagenesis to up-regulate, over-express, and/or increase, the production of enzymes. Site directed mutagenesis comprises the mutation of a DNA molecule at specific nucleic-acid base-pair sequence. Site directed mutagenesis may occur in the chromosomal DNA, or in extra-chromosomal DNA, from a vector. Additionally, the site-directed mutagenesis may comprise gene deletion/excision, primer mediated mutagenesis, cassette mutagenesis, add-on mutagenesis, mismatch mutagenesis, gene conversion, topological manipulation, specialized recombination, or PCR-mediated mutagenesis. Further, mutagenesis and over-expression of a gene may be induced by any mutagen. For instance, ionizing radiation, UV exposure, deamination, intercalation, alkylation, analog insertion, transposon multiplication, and other molecular biology techniques may be used, without limitation. In certain instances, mutagen exposure induces the microorganism to acquire a vector, such as a plasmid. Further, mutagenesis may induce the incorporation of vector DNA into the chromosomal DNA. For the purpose of this disclosure, directed, mutagenic technique may be implemented in order to induce additional production of an enzyme. Further, the mutagenesis may be used to increase the activity of the enzyme.

[0037] In certain instances, the enzymes are chemically modified after production. The enzymes may be modified prior to or after harvesting from the microorganisms. Any process known to one skilled in the art may be implemented, such as but not limited to addition of functional groups, addition of other peptides, altering the chemical nature, and/or structural changes. For example, processes like acylation, glycosylation, ubiquitination, deamidation, and/or cleavage, are envisioned to have applications within the present disclosure, without limitation.

[0038] The produces and modified enzymes are utilized in biochemical liquor. The biochemical liquor comprises a liquid mixture of proteins, enzymes, inorganic catalysts, and organic and inorganic compounds. The biochemical liquor further comprises salts, electrolytes, metals, and/or other molecules that aid, improve, or alter the operations of an enzyme broth, or biochemical liquor. The biochemical liquor is a mixture of at least one of each of the aforementioned groups without limitation. The biochemical broth may be suspended in any known solvent; preferably organic solvents. In exemplary embodiments, water is the solvent.

[0039] Process. As illustrated in FIG. 1, the process comprises a flow of coal through a sequence of individual treatment steps. In STAGE I, the process comprises at least one pretreatment step. The coal then undergoes solubilization, which may be included in the pretreatment steps. The pretreated, solubilized coal material is converted to liquid hydrocarbons. Simultaneously, or sequentially, the material may undergo sulfur and nitrogen conversion. Removing sulfur and nitrogen from the product improves performance of the fuel after processing, such as separation. The different fuels are separated, such that aqueous phase reactants are recycled, and/or the wastewater is treated for return to the system. In STAGE II, the hydrocarbon phase products from the conversion step are further refined. The refining of the products comprises fuel refining, enrichment, and distillation to improve product qualities.

[0040] Pretreatment. The pretreatment step comprises a physical and chemical degradation of the coal, to produce a degraded coal. In order to increase efficiency of the disclosed process it is advantageous to increase the surface area of the material. The surface area of the coal may be increased by reducing the particle volume, such as in the process of comminution. For instance, surface coal, mined coal, coal tailings, or remaindered coal is mechanically broken down or crushed into a fine particulate. Remaindered coal may comprise, without limitation, coal sourced from any industry from which it was rejected for use. The particulate may comprise pebbles, dusts, powders, or the like without limitation.

[0041] In certain instances, the coal is treated in-situ, for instance, in an underground coal seam, by high-pressure steam. Underground wells, conduits, and/or other lines deliver steam to a coal mine. The steam may be pressurized, superheated, or combinations thereof in order to increase penetration into the seam. The steam is used to fracture the coal bed underground prior to pretreatment. Further, the high-pressure steam mechanically separates the coal from surrounding rock formations.

[0042] Additionally, the coal is subjected to a chemical treatment. Chemical treatment of the coal increases the reactivity of the coal. Additionally, chemical treatment is designed to remove, digest, or eliminate non-coal materials from the coal products. Further, the chemical treatment may increase the surface area of the coal further by inducing the

formation of pores, cavities, pits, and the like. The chemical treatment may compromise an oxidative agent. In certain embodiments, mild ionic, acid, base, or free radical solutions are applied to oxidize the coal. In an embodiment, the solution comprises a mild acid solution. In an exemplary embodiment, the chemical treatment comprises hydrogen peroxide. The hydrogen peroxide is in concentrations between about 10% and about 50%; alternatively between about 20% and about 40%, and in preferably about 30% hydrogen peroxide. As discussed above, the solutions are injected into coal mines with steam, or water, therefore it is preferable the solutions are aqueous. In certain instances, an inorganic chemical treatment may be implemented.

[0043] Solubilization. Further, the pretreatment steps comprise solubilization of the degraded coal. Once mechanically disintegrated, and chemically oxidized, a first set of enzymes is introduced to break the cross-linking bonds in coal. In certain instances, the first enzymes may be derived from enzymes found, for example, in the genera *Peoria*, *Polypore*'s, genera *Poria*, *Polyporus*, without limitation. The first enzymes allow the coal particles to dissolve into the liquid medium. In embodiments, the liquid medium is the same as is used to deliver the enzymes. In certain instances, the medium is the biochemical liquor described above. Further, the liquid medium comprises an aqueous medium. The solubilized coal particles are suspended in the liquid medium forming a coal slurry; alternatively, a coal suspension, a coal mixture, a coal colloid, or a coal solution, without limitation. The coal slurry improves accessibility to the coal particles by the enzymes from the biochemical liquor. Suspending the particles in the medium may improve reaction kinetics during the subsequent enzymatically mediated steps. The coal slurry further improves transfer of the coal between processing steps.

[0044] Conversion. After solubilization, the coal is processed by conversion steps. In certain instances, the solubilized coal in the coal slurry is converted to smaller or lower hydrocarbons. The lower hydrocarbons may comprise any hydrocarbon, for instance hydrocarbons comprising between about 24 carbons and about 2 carbons. The second enzyme, or second enzyme solution, is maintained in the biochemical broth. The second enzyme solution may be derived from enzymes produced by microorganisms for the genera, *Thiobacillus*, *Candida*, *Streptomyces*, *Pseudomonas*, *Penicillium*, *Trichoderma*, for example, without limitation. The second enzyme may be introduced to the coal slurry during the conversion process. In certain instances, the exposure of the coal slurry to a second enzyme comprises converting coal to lower molecular weight hydrocarbon fractions. Alternatively, the second enzyme is a converting enzyme. Converting enzymes are those selected, engineered, or modified to catalytically convert large hydrocarbon molecules found in coal to lower molecular weight hydrocarbons. During the conversion step, the hydrocarbons undergo saturation, and sulfur, nitrogen, and other contaminant conversion. The conversion step forms a reaction slurry, or a hydrocarbon slurry with the biochemical liquor.

[0045] The enzymatic conversion reactions successively break the native, original, or solubilized, coal particles into smaller hydrocarbon molecules that remain in the reaction slurry. The enzymatic conversion reactions convert the high molecular weight molecular components of coal to lower molecular weight mixtures of hydrocarbon liquids and hydrocarbon gases.

[0046] During conversion, certain waste products, contaminants, and potential pollutants are removed from the process. In certain instances, the removal of these products is mediated by a third enzyme added to the reaction slurry. The third enzyme, or third enzyme solution, reacts with the products of catalytic conversion to liberate sulfur from the hydrocarbon complexes and form a variety of simpler sulfur-containing compounds, which are soluble in the reaction slurry. The soluble sulfur-containing compounds may be filtered from the reaction slurry and processed for other products.

[0047] Additionally, in order to remove other waste products, contaminants, and potential pollutants, a fourth enzyme may be added to the reaction slurry. In certain instances, any number of waste removal enzymes may be used to specifically eliminate, sequester, or cleave the unwanted compounds. In certain embodiments, the fourth enzyme solution reacts with the products of catalytic conversion to liberate nitrogen and form a variety of simpler nitrogen-containing compounds, which are soluble in reaction slurry.

[0048] The processed wastes may comprise gases solubilized in the reaction slurry. Gases may comprise nitrogen, oxides of nitrogen, sulfur, oxides of sulfur, carbon monoxide, carbon dioxide, and other gases without limitation. Certain waste products are used for further processes, such as syngas production, or catalyzed synthesis of liquid fuels. Further, enzymatically-catalyzed reactions convert the complex sulfur and nitrogen compounds found in coal to simpler forms that are removed during product separation.

[0049] As understood by one skilled in the art, the reaction properties such as temperature, pressure, pH and residence time are differentially monitored, and controlled for maximized production. In certain instances, the reaction properties are controlled to obtain a distribution of hydrocarbon molecular weights in the product stream. Further, as the reaction slurry comprises the biochemical liquor, altering the conditions may optimize conversion.

[0050] As discussed hereinabove, the biochemical liquor comprises any number of enzymes. As understood by one skilled in the art, each enzyme has preferred conditions for efficient catalysis. As such, cycling the reaction conditions, such as temperature and pressure, is envisioned to maximize the efficiency of any portion of the process, or the action of any portion of the enzymes. In other embodiments, the conversion step consists of separate reaction vessels for the solubilization, catalytic cracking, and nitrogen and sulfur conversion reactions. Such an arrangement permits different operating conditions to be used in each vessel, such as temperature and individual reactor recycle rates, to optimize the enzyme-catalyzed reactions.

[0051] Product Separation. Following conversion, the hydrocarbon mixture is sent into settling tanks. In embodiments, the settling tank may be any vessel configured for separating the hydrocarbon liquid from the aqueous coal slurry. In certain instances, the settling tank may comprise a dynamic settler, wherein a constant low volume, or slow velocity, stream of the reaction slurry is introduced to separate the aqueous and hydrocarbon phases. Alternatively, the settling tank comprises a static settler, where the aqueous phase coal slurry settles from the lighter hydrocarbons by virtue of gravity. In certain embodiments, the remaining sulfur and nitrogen compounds distribute to the water phase. The hydrocarbon phase is separated by drawing off the lighter

hydrocarbon layer from the denser aqueous layer. Alternatively, a conduit withdraws the aqueous phase from the bottom of the tank.

[0052] Product Enrichment. The hydrocarbon layer, which already contains gasoline, kerosene, diesel, and fuel oils is sent to Stage II where it is further upgraded by converting the lower-valued fractions, naphtha, diesel, fuel oils, waxes, and the like to higher-valued fractions such as gasoline or kerosene. In embodiments, the product enrichment may comprise enzymatic conversion, molecular photofragmentation, conversion, and enzyme assisted molecular photo-fragmentation conversion. In certain instances, the product enrichment comprises a fifth enzyme, or fifth enzyme solution introduced to the hydrocarbon products from the separation step. Following enrichment, the hydrocarbon mixture is separated into final products by conventional distillation.

[0053] Ex-situ Processing. FIG. 2 illustrates an embodiment of processing of ex-situ coal feedstocks continuously, or an EX-system 10. In the EX-system 10, pretreatment, conversion, and product processes are designed to be in fluid communication. In EX-system 10, the coal is first ground into small particles by mechanical means 12. As previously described, the mechanical means 12 creates a particulate product stream 14. The particulate product stream is introduced to chemical treatment system 16, comprising, for example, a weak acid solution. In certain instances, mechanical means 12 and chemical treatment system 16 may comprise a single vessel, or single processing facility. The coal and acid solution form coal slurry where the coal undergoes pre-oxidation. The extent of the pre-oxidation is determined by the residence time of the slurry in the chemical treatment system 16. Further, in combined embodiments, the oxidation of the coal in the coal slurry may be controlled, at least in part, by the degree of agitation provided by mixers 18.

[0054] The slurry product stream 20 is then pumped out of the feed tank and into a reactor stage 22. The reactor stage 22 comprises the solubilization reaction. In certain instances, the first enzyme stream 24, with enzymes selected for solubilizing the coal, is injected into slurry product stream 20. Alternatively, first enzyme stream 24 is injected directly into reactor stage 22. Without wishing to be limited by theory, it may be beneficial for first enzyme stream 24 to be introduced to slurry stream 20 prior to introduction to reactor stage 22.

[0055] Reactor stage 22 comprises the enzyme mediated catalytic conversion reaction. Second enzyme stream 26 is injected into reactor stage 22. The enzymes react catalytically, convert the large hydrocarbon molecules, and produce product stream 30. Further, third enzyme stream 27 to convert sulfur compounds and fourth enzyme stream 28 to convert the nitrogen compounds are added to reactor stage 22. The third enzyme stream 27 and fourth enzyme stream 28 convert the respective contaminants found in the coal slurry 20 into simpler, water-soluble forms. Reactor stage effluent 23 is continuously split between a recycle stream 25, which is pumped back into the reactor stage 22, and a product stream 30.

[0056] In other embodiments, the reaction stage 22 consists of separate reaction vessels for the solubilization, catalytic conversion, and nitrogen and sulfur conversion reactions. A multiple reactor arrangement permits different operating conditions to be used in each vessel, such as temperature and individual reactor recycle rates, to optimize the different suites of reactions.

[0057] The product stream 30 is pumped into the separation stage 32. Separation stage 32 may comprise gas vent 33 for

withdrawing the gases and volatile compounds released during separation. In certain instances, gas vent **33** vents some gases that were dissolved in product stream **30**. The separation stage **32** comprises the step where the aqueous phase **36** settles out under the hydrocarbon phase **34**. Separation stage **32** comprises a settler, or settling vessel. Alternatively, separation stage is a filter or other apparatus to separate aqueous and hydrocarbon phases from product stream **30**. Separation stage **32** comprises a continuous flow, oil separation vessel. The aqueous phase **36** is withdrawn from separation stage **32** and routed via recycle stream **39** to the reactor stage **22**. The recycle stream comprises a wastewater treatment system. The treatment system comprises any system configured as a sour water treatment, configured to remove residuum, as well as nitrogen and sulfur by-products. Treated water is then recycled back to the reactor section.

[0058] In certain instances, the hydrocarbon phase **34** may be withdrawn from the top of the settler as hydrocarbon stream **38** for enrichment and/or distillation to produce transportation fuels. The hydrocarbon stream **38** is pumped to a product enrichment stage.

[0059] In-Situ Processing Another embodiment involves the continuous processing of in-situ coal, or IN-system **100**, which is illustrated in FIG. **3**. In this embodiment, the IN-system **100** comprises an abandoned, collapsed, inaccessible, or otherwise difficult to mine coal deposit, or underground coal seam **101**. In embodiments, at least one well **102** is drilled into the coal seam **101**. The wells **102** are generally configured for the transport of liquids and slurries between the underground coal seam **101** and the processing center **120**. Further, the wells **102** may be configured for the continuous circulation of process fluids in and out of the underground coal seam **101**. It can be envisioned that a plurality of wells **102** would improve product yield, processing time, and the general economics of the IN-system **101**, without limitation.

[0060] The IN-system **100** process begins with the injection of steam in the well **102** conduits to induce fracturing of the underground coal seam **101**. This fracturing step **105** is configured to break the coal seam into particulates, coal gravel, or the like. In certain instances, using high-pressure steam is according to conventional practices of the underground coal mining industry.

[0061] After the fracturing step **105** is complete, the high-pressure steam is withdrawn. The oxidation step **106** comprises injecting an acid solution to pre-oxidize the fractured coal. In embodiments, the acid solution is recycled continuously to form a circulating process stream **150**, such that the acid is pumped into well **102A** at one side of the seam, pumped out of well **102B** on the other side of the seam. To complete the cycle, the acid solution is transported back and pumped into the first well **102A**. The circulating process stream **150** may be repeated until the desired level of pre-oxidation is achieved.

[0062] As described above, the solubilization step **107** may comprise the introduction of the first enzyme solution into the underground coal seam **101**. The first enzyme solution is introduced into the circulating process stream **150** to solubilize the exposed coal into the circulating process solution.

[0063] Once an adequate level of soluble coal is achieved, to create coal slurry in the circulating process stream **150**, the additional enzymes are added either sequentially or simultaneously. In certain instances, the second enzyme **106**, third enzyme **107**, and fourth enzyme **108** solutions are added to

the circulating stream **150**. As described herein above, the second enzyme stream **106** is selected to catalytically crack the hydrocarbons. The third enzyme **107** and fourth enzyme **108** solutions are selected to remove sulfur and nitrogen-containing compounds and/or waste products from the circulating process stream **150**. In embodiments, further enzyme streams may be injected into circulating process stream **150** to optimize the solubilization and conversion of the coal.

[0064] A portion of the circulating process stream **150** is then split and taken as a raw product stream **160** and sent to a processing stage **120**. The processing stage **120** is similar to the one used in ex-situ embodiments of the process described above. Separation vessel **162** comprises the step where the aqueous phase **163** settles out under the hydrocarbon phase **164**. Separation vessel **162** comprises a settler or settling vessel. The aqueous phase **163** is withdrawn from separation vessel **162** and routed via recycle stream **170** to the circulating process stream **150**. The recycle stream **170** comprises a wastewater treatment system. The treatment system comprises any system configured for sour water treatment, configured to remove residuum, as well as nitrogen and sulfur by-products. In certain instances, the hydrocarbon phase **164** may be withdrawn from the top of the settler as hydrocarbon stream **168** for enrichment and/or distillation to produce transportation fuels. The hydrocarbon stream **38** is pumped to a product enrichment stage.

[0065] Mutagenesis. Methods used to produce suitable enzymes for implementation in Stage II for fuel upgrade include using catalytic antibodies. As illustrated in FIG. **4**, biological enzymes are identified for the catalytic processes desired. In certain instances, the biological enzymes are derived from the microorganisms discussed herein above. In further embodiments, the enzymes comprise Mother Nature only (MNO) enzymes. MNO enzymes are the phenotypic expressions of unmodified genetic sequences within the microorganisms. Alternatively, MNO enzymes are wild-type enzymes. In further instances, illustrated in FIG. **5**, the MNO enzymes are selected from those that comprise an activated enzyme. In certain instances, the activated enzyme screening is conducted by an antibody assay. Alternatively, any suitable screening method may comprise any suitable protocol to identify the wild type MNO enzymes, as further illustrated in FIGS. **6** and **7**.

[0066] The MNOs selected are formed by directed evolution, as illustrated in FIG. **8**. The selected MNOs are subject to site-directed and random mutagenesis throughout the enzyme, not solely restricted to the active site. In certain instances, the enzymes are also subject to mutagenesis at allosteric sites, and at sites remote from active and/or allosteric sites. The mutagenesis at multiple sites comprises a means to both promote and restrict potential products as illustrated in FIGS. **9** and **10**. In certain instances, the mutagenesis includes active site chemical redesign as shown in FIG. **11**. Preferably, the results include a rational design enzyme, or enzymatic structure.

[0067] The structure is synthesized, computationally designed, with motifs attached to enzyme scaffolds. As enzymes are rather large molecules, having hundreds of amino acids, tens of kilo Daltons (Kds), and thousands of cubic angstroms, they may be considered spatially inefficient. In certain instance, large enzyme molecules comprise small active sites. Enzymatic reactive sites are quite small by comparison and the other folded amino acids serve as a scaffolding to create the reactive site volume. These "other" amino

acids can be, relatively speaking, quite far from the active site of the enzyme as illustrated in FIG. 12. Additionally, the enzymes may include cofactor attachment site redesigns, shown in FIG. 13. In order to induce cofactor attachment site redesigns the implementation of site directed mutagenesis are repeated as discussed hereinabove, for example, paragraph 21.

[0068] As diagrammed in FIG. 14, a shaped IR femtosecond laser pulse may be impinged upon the enzymatic complex to induce reactant fragmentation. Further, it can be envisioned that any particular impinging radiation, known to one skilled in the art, may be capable of the same reactant fragmentation, without limitation. The laser pulse for directed fragmentation of reactants/conversion to products may aid the formation of reactant products. As understood by one skilled in the art, multiple fragmentation reactant products may be formed. In certain instances, the multiple fragmentation products may be advantageous for the formation of a range of reactant fragments. Alternatively, the shaped IR femtosecond laser pulses in conjunction with above mentioned enzyme techniques to assist in selective fragmentation of reactants at enzymatic active sites, allosteric sites, and sites remote from binding or allosteric sites as shown in FIG. 15. As understood by one skilled in the art, the bonding of the reactant, hydrocarbon, molecular to the enzyme reactive site may comprise a covalent, non-covalent, hydrogen, ionic, Van der Waals, or other bond, interaction, coupling, or association, without limitation. Further, the enzyme reactive site is configured to restrict the reactant molecule, and its range of movement. Further, the reactive site restricts internal degrees of freedom, to favorably target the femtosecond laser pulses to the preselected internal bond. In certain instances, the enzyme reactive site damps the internal degrees of freedom, such that internal vibrational rearrangement (IVR) is prevented, and the laser energy is focused to the preselected internal bond.

[0069] While the preferred embodiments of the invention 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 invention. The embodiments described and the examples provided herein are exemplary only, and are not intended to be limiting. Many variations and modifications of the invention disclosed herein are possible and are within the scope of the invention. Accordingly, the scope of protection is not limited by the description set out above, but is only limited by the claims that follow, the scope including all equivalents of the subject matter of the claims.

We claim:

1. A method for producing liquid hydrocarbon product comprising,

disintegrating a hydrocarbon source;
treating the disintegrated hydrocarbon source chemically;
solubilizing the disintegrated hydrocarbon source to form a slurry;
admixing a biochemical liquor into the slurry, wherein the biochemical liquor comprises at least one conversion enzyme, to form liquid hydrocarbons;
separating liquid hydrocarbons from the slurry, wherein contaminants remain in the slurry; and
enriching the liquid hydrocarbons to form a liquid hydrocarbon product.

2. The method of claim 1, wherein disintegrating the hydrocarbon source comprises comminution of the hydrocarbon source.

3. The method of claim 2, wherein comminution comprises grinding.

4. The method of claim 2, wherein comminution comprises high-pressure steam treatment.

5. The method of claim 1, wherein treating the disintegrated hydrocarbon source further comprises oxidation of the hydrocarbon source.

6. The method of claim 1, wherein solubilizing the disintegrated hydrocarbon source comprises treating the disintegrated hydrocarbon source with at least one enzyme.

7. The method of claim 1, wherein admixing biochemical liquor comprises admixing at least one additional enzyme.

8. The method of claim 7, wherein admixing at least one additional enzyme further comprises admixing an enzyme for converting a hydrocarbon source to lower molecular weight hydrocarbons.

9. The method of claim 1, wherein separating liquid hydrocarbons comprises a process of settling the slurry from the liquid hydrocarbon.

10. The method of claim 1, wherein separating liquid hydrocarbons comprises settling contaminants from the liquid hydrocarbon.

11. The method of claim 1, wherein enriching the liquid hydrocarbon comprises admixing the liquid hydrocarbon with at least one enzyme.

12. The method of claim 1, wherein the hydrocarbon source comprises at least one selected from the group consisting of coal, anthracite coal, bituminous coal, lignite, sub-bituminous coal, low-rank coals, coke, tar sand, oil shale, and combinations thereof.

13. The method of claim 1, wherein the biochemical liquor comprises a modified enzyme.

14. The method of claim 13, wherein the modified enzyme comprises an enzyme that is genetically modified.

15. The method of claim 13, wherein the modified enzyme comprises an enzyme that is chemically modified.

16. The method of claim 13, wherein the modified enzyme comprises an enzyme configured for bond selective photofragmentation.

17. The method of claim 1, wherein the method is conducted in-situ in a coal mine.

18. The method of claim 1, wherein the method is conducted ex-situ on mined coal.

19. The method of claim 1, wherein enriching the liquid hydrocarbons comprises improving the liquid hydrocarbon product qualities prior to distillation.

20. The method of claim 1, wherein the liquid hydrocarbon product comprises at least one selected from the group consisting of gasoline, diesel, kerosene, and distillates thereof.

21. A method for in-situ coal to liquid hydrocarbon conversion, comprising:

locating an underground coal seam;
drilling at least one well, the well in contact with the underground coal seam and having a means to cycle liquids therethrough;
pressurizing the underground coal seam with steam;
cycling reactants through the underground coal seam, wherein the reactants comprise at least one enzyme, to form a slurry;
withdrawing a portion of the slurry;
processing the slurry to produce the liquid hydrocarbon;
separating the liquid hydrocarbon from the slurry; and
returning the slurry to the coal seam for further processing.

22. The method of claim **21**, wherein the at least one well is in fluid communication with a reactant stream.

23. The method of claim **21**, wherein the at least one well is in fluid communication with a slurry processing stream.

24. The method of claim **21**, wherein cycling reactants to form a slurry further comprises

solubilizing the coal to form a slurry;

converting the coal to form liquid hydrocarbons;

separating contaminant compounds from the liquid hydrocarbons, wherein the contaminant compounds comprise pollutants;

settling the slurry from the liquid hydrocarbons, wherein the liquid hydrocarbons are suitable for liquid fuels; and processing the liquid hydrocarbons to liquid fuels.

25. The method of claim **24**, wherein the step of solubilizing the coal comprises treating the coal with at least one enzyme.

26. The method of claim **24**, wherein the step of converting the coal comprises treating the coal with at least one enzyme.

27. The method of claim **24**, wherein the step of separating contaminant compounds comprises treating the liquid hydrocarbons with at least one enzyme.

28. A method for using an enzyme to produce liquid fuels, comprising

selecting a microorganism, the microorganism producing an enzyme;

modifying a microorganism genetically, to increase the production of the enzyme;

modifying the enzyme structurally, to alter the activity of the enzyme, to form a modified enzyme;

collecting the modified enzyme, to form a biochemical liquor comprising at least one modified enzyme; and

exposing a hydrocarbon source to the biochemical liquor to form a liquid fuel precursor.

29. The method of claim **28**, wherein the step of selecting a microorganism comprises selecting at least one microorganism chosen from the group consisting of hypoliths, endoliths, cryptoliths, acidophiles, alkaliphiles, thermophiles, lithoautotrophs, halophiles, piezophiles, and combinations thereof.

30. The method of claim **28**, where modifying a microorganism comprises inserting a nucleic acid vector.

31. The method of claim **28**, wherein modifying a microorganism genetically comprises directed mutagenesis.

32. The method of claim **28**, wherein modifying an enzyme comprises structurally changing an enzyme.

33. The method of claim **28**, wherein exposing the biochemical liquor to the hydrocarbon source further comprises transmitted-radiation directed fragmentation.

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