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(54) Title: COMPOSITIONS AND METHODS FOR REDUCING MINING CARBON EMISSIONS

(57) Abstract: The subject invention provides compositions and methods for reducing atmospheric carbon dioxide, methane, and/or nitrous oxide emissions during mining and refining processes. In preferred embodiments, a composition comprising a beneficial microorganism and/or a growth by-product thereof is contacted with mined substance before and/or during leaching and/or flotation processes. The composition is capable of increasing the efficiency of leaching and reducing the amount of grinding needed before flotation, thus reducing the amount of emissions produced from the refining process.



COMPOSITIONS AND METHODS FOR REDUCING MINING CARBON EMISSIONS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application No. 63/508,608, filed June 16, 2023, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Gases that trap heat in the atmosphere are called “greenhouse gases,” or “GHG,” and include carbon dioxide, methane, nitrous oxide and fluorinated gases (EPA report 2016 at 6).

Carbon dioxide (CO₂) enters the atmosphere through burning fossil fuels (coal, natural gas, and oil), solid waste, trees and wood products, and also as a result of certain chemical reactions, e.g., the manufacture of cement. Carbon dioxide is removed from the atmosphere by, for example, absorption by plants as part of the biological carbon cycle.

Nitrous oxide (N₂O) is emitted during industrial activities and during combustion of fossil fuels and solid waste. In agriculture, over-application of nitrogen-containing fertilizers and poor soil management practices can also lead to increased nitrous oxide emissions.

Fluorinated gases including, e.g., hydrofluorocarbons, perfluorocarbons, sulfur hexafluoride, and nitrogen trifluoride are synthetic, powerful greenhouse gases that are emitted from a variety of industrial processes.

Methane (CH₄) is emitted during the production and transport of coal, natural gas, and oil. Furthermore, other agricultural practices, and the decay of organic waste in lagoons and municipal solid waste landfills can produce methane emissions.

Based on recent measurements from monitoring stations around the world and measurement of older air from air bubbles trapped in layers of ice from Antarctica and Greenland, global atmospheric concentrations of GHGs have risen significantly over the last few hundred years (EPA report 2016 at, e.g., 6, 15).

Especially since the Industrial Revolution began in the 1700s, human activity has contributed to the amount of greenhouse gases in the atmosphere by burning fossil fuels, cutting down forests, and conducting other activities. Many greenhouse gases emitted into the atmosphere remain there for long periods of time ranging from a decade to many millennia. Over time these gases are removed from the atmosphere by chemical reactions or by emissions sinks, such as the oceans and vegetation that absorb greenhouse gases from the atmosphere. Because each greenhouse gas has a different lifetime and a different ability to trap heat in the atmosphere and in order to be able to compare different gases, emissions are generally converted into carbon dioxide

equivalents using each gas's global warming potential, which measures how much a given amount of the gas is estimated to contribute to global warming over a period of 100 years after being emitted.

Based on these considerations, the EPA determined that the heating effect caused by greenhouse gases, also termed "radiative forcing," has increased by about 37% since 1990 (*Id.* at 5 16).

Although global emissions of all major greenhouse gases increased between 1990 and 2010, the net emissions of carbon dioxide, which accounts for about three-fourths of the total global emissions, increased by 42%, whereas emissions of methane increased by about 15%, emissions of fluorinated gases doubled, and emission of nitrous oxide emissions increased by about 9% (*Id.* at 10 14).

World leaders have attempted to curb the increase of GHG emissions through treaties and other inter-state agreements. One such attempt is through the use of carbon credit systems. A carbon credit is a generic term for a tradable certificate or permit representing the right to emit one ton of carbon dioxide, or an equivalent GHG. In a typical carbon credit system, a governing body sets 15 quotas on the amount of GHG emissions an operator can produce. Exceeding these quotas requires the operator to purchase extra allowances from other operators who have not used all of their carbon credits.

One goal of carbon credit systems is to encourage companies to invest in more green technology, machinery and practices in order to benefit from the trade of these credits. Under the 20 Kyoto Protocol of the United Nations Framework Convention On Climate Change (UNFCCC), a large number of countries have agreed to be bound internationally by policies for GHG reduction, including through trade of emissions credits. While the United States is not bound by the Kyoto Protocol, and while there is no central national emissions trading system in the U.S., some states, such as California and a group of northeastern states, have begun to adopt such trading schemes.

25 Because global warming may contribute to steeper temperature fluctuations, increased global precipitation, flooding and droughts, as well as changes in sea surface temperature and sea levels, there exists a need to reduce greenhouse gases to slow these detrimental effects.

BRIEF SUMMARY OF THE INVENTION

30 The subject invention provides compositions and methods for reducing greenhouse gas emissions using additives in mining and refining processes. In preferred embodiments, a composition comprising one or more microorganisms and/or one or more microbial growth by-products is contacted with an ore, gangue, or other substance containing a substance of interest, such as, for example, a metal, mineral, or element. The composition is capable of, for example, reducing

the use of fossil fuels, and thus, reducing the amount of greenhouse gas emissions produced from mining and subsequent transportation or refining processes.

Advantageously, the compositions and methods of the subject invention increase the efficiency of various mining and refining processes, including, for example flotation and leaching. Accordingly, the subject invention can be useful for increasing the efficiency and reducing the energy needed for mining, transportation, and/or refining. In certain embodiments, the subject compositions and methods decrease greenhouse gas emissions in mining or industrial processes by, for example, reducing the amount of grinding necessary during refining or increasing the rate of leaching.

In certain embodiments, the subject invention provides a bioleaching composition that can be employed in a method of recovering metals.

In certain embodiments, the bioleaching composition comprises a metal solubilizing agent, such as, for example, an acid-oxidant mixture or a metal-solubilizing microorganism.

The acid can be any acid that can solubilize an insoluble metal salt, such as, but not limited to, sulfuric acid, hydrochloric acid, nitric acid, phosphoric acid, and/or hydrocyanic acid (or a salt thereof). The acid can be used in a wide range of concentrations, from less than one percent to more than ten percent.

The oxidant can be selected from, for example, ferric chloride, chlorine, bromine, and oxygen. The oxidant can be used in sufficient quantity to liberate the target metal as a soluble salt but at quantities that minimize oxidant waste.

In certain exemplary embodiments, the bioleaching composition comprises a biosurfactant, an acid and an oxidant, wherein the biosurfactant is a sophorolipid, the acid is sulfuric acid, and the oxidant comprises Fe^{2+} or Fe^{3+} .

In certain embodiments, the subject invention provides methods for liberating a target metal from a source of said target metal, wherein a bioleaching composition of the subject invention is applied to the metal source. The metal source can be, for example, ore, mine tailings, slag and/or electronic waste from, for example, batteries and circuits.

In certain embodiments, the target metal is liberated as a soluble salt and/or a significant portion of any matrix that entraps the target metal is removed, thereby facilitating the isolation of the target metal from a non-soluble portion. For example, the bioleaching composition can be combined with a particulate ore that contains at least one target metal. The components can be mixed for reaction of the ore and at least one component of the bioleaching composition, such that a soluble metal-comprising solution and a non-solution phase is formed. In some embodiments, the particulate metal source is a fine powder. The particles in the fine powder can be less than about 1 m, about 75 cm, about 50 cm, about 25 cm, about 10 cm, about 75 mm, about 50 mm, about 25 mm,

about 10 mm, about 5 mm, about 1 mm, about 100 μm , about 10 μm , about 1 μm , about 100 nm, about 10 nm, or about 1 nm in diameter. In preferred embodiments, the subject compositions enable efficient leaching of larger particles, relative to particles that have not been contacted to the subject compositions.

5 Mixing of the bioleaching composition with the metal source can be performed via, for example, stirring, bubbling of a reactive or inert gas, ultrasonic mixing, piezoelectric agitation, or any combination thereof.

10 In certain embodiments, the solution comprising the soluble metal can be separated from the non-solution phase by, for example, decantation, filtration, centrifugation, or any combination thereof.

15 In some embodiments, methods of extracting a target metal from ore are provided wherein an ore in a particulate state is combined with a microorganism and/or bioleaching composition according to the subject invention to form a liquid slurry. The microbes can be live (or viable), or, preferably, inactive at the time of application. In certain embodiments, the microorganisms can grow *in situ* and produce active compounds (e.g., metabolites) that can directly or indirectly solubilize metals.

20 The slurry can then be left and/or agitated for any amount of time sufficient to leach the target metal from the ore, as described in U.S. Application Serial Number 18/249,244, which is hereby incorporated by reference in its entirety. The slurry can optionally be mixed and/or circulated continuously (e.g., mechanically or using aeration) throughout the leaching time period to ensure that maximum contact is made between the ore particles and the bioleaching composition.

25 Advantageously, the compositions and methods of the subject invention can be useful for liberating a wide variety of target metals including, but not limited to, aluminum, arsenic, cerium, cesium, chromium, cobalt, copper, dysprosium, erbium, europium, gadolinium, gallium, germanium, gold, hafnium, holmium, indium, iridium, lanthanum, lead, lithium, lutetium, magnesium, manganese, mercury, neodymium, nickel, niobium, palladium, platinum, praseodymium, rhodium, rubidium, ruthenium, samarium, scandium, silver, tantalum, tellurium, terbium, thallium, tin, titanium, tungsten, uranium, vanadium, ytterbium, yttrium, zinc, zirconium, any mixture thereof or any other rare earth or transition metal.

30 Advantageously, in certain embodiments, the bioleaching composition and methods of the subject invention are effective at standard leaching temperatures known in the metallurgical arts. In certain embodiments, the bioleaching composition and methods are useful at lower than standard temperatures, for example, at about 60°C to 90°C. Thus, the subject invention provides methods of reducing the energy requirements for metal extraction and leaching while producing similar or
35 improved yields.

Furthermore, in certain embodiments, the subject invention provides methods of enhancing the performance of standard leaching reagents, e.g., acids, oxidants and/or cyanide, wherein the reagents are applied alongside a biosurfactant and/or a biosurfactant-producing microbial culture according to the subject invention. Advantageously, in certain embodiments, the biosurfactant(s) of the subject bioleaching composition work in synergy with the leaching reagents, e.g., the acid and the oxidant, to improve their performance. This not only allows for potentially reduced volume usage of harsh leaching reagents, but also enhanced leaching yields with reduced time and/or energy expenditure, thereby reducing greenhouse gas emissions.

In some embodiments, the metal source, such as, for example, ore, mine tailings, slag and/or electronic waste from, for example, batteries and circuits, can be treated with a pre-leaching composition before the leaching process. In certain embodiments, the method can further comprise, after obtaining the metal source, concurrent with the treatment by pre-leaching compositions of the subject invention, and/or after treating the metal source with the pre-leaching composition, subjecting the metal source to one or more beneficiation processes. The one or more beneficiation processes can include, for example, comminution, scrubbing, washing, screening, flotation, and/or hydrocycloning. In certain embodiments, the pre-leaching composition comprises a microbial biosurfactant. In certain embodiments, the composition comprises biosurfactants, and, optionally, other compounds, such as, for example, surfactants, pine oils, xanthates, or any combination thereof.

In certain embodiments, the pre-leaching composition according to the subject invention is effective due to amphiphile-mediated penetration of the metal source. In some embodiments, the sophorolipid or other biosurfactant serves as a vehicle for facilitating the transport of metal. For example, in some embodiments, a sophorolipid will form a micelle containing the metal, wherein the micelle is less than about 100 μm , less than about 10 μm , less than about 1 μm , less than about 100 nm, less than about 50 nm, less than about 25 nm, less than about 15 nm or less than about 10 nm, less than about 5 nm, or less than about 2 nm in size. The small size and amphiphilic properties of the micelle allow for enhanced penetration into the metal source so that greater contact can be made with metal therein.

In preferred embodiments, the method comprises flotation of the target metals pre-leaching composition according to the subject invention, which facilitates the attachment of air bubbles to the target metal so that it floats to the surface of the liquid during aeration. At the surface, the target metals are supported by a froth layer and then collected. Unattached materials remain submerged in the liquid below. Advantageously, in certain embodiments, the biosurfactant modifies the surface properties of the target metal, selectively binding to the surface and imparting hydrophobicity to the metal to facilitate the attachment of air bubbles. In certain embodiments, the methods enable larger particles to be floated relative to target metal particles that have not been treated with the subject

compositions, thereby reducing greenhouse gas emissions produced to decrease particle size by, for example, grinding a mined target metal.

In certain embodiments, methods are also provided for separating and/or extracting valuable minerals and/or metals, such as, e.g., gold, silver, platinum, and rare earth metals, from rock, ore, 5 tailings, and/or coal combustion waste products. Advantageously, these compositions and the methods of their use provided herein, are safe, environmentally-friendly, and cost-efficient alternatives to traditional chemical reagents utilized in froth flotation or performance enhancers that can be added to traditional chemical reagents utilized in froth flotation.

10 In certain preferred embodiments, the subject invention provides a beneficiation composition for use in metal and mineral froth flotation, wherein the composition comprises a microbial culture and/or a microbial growth by-product. In certain embodiments, the microbial growth by-product is a biosurfactant, either in crude form or purified form. The biosurfactant may also be chemically modified. In some embodiments, the composition comprises an aqueous carrier.

15 In certain embodiments, the composition can be used in methods of direct flotation and reverse flotation, as described in U.S. Application Serial Number 18/253,957, which is hereby incorporated by reference in its entirety. In direct flotation processes, the floated particles (the concentrate) is the beneficiary and the tailings are the gangue. In reverse flotation processes, the gangue constituent is floated into the concentrate and the beneficiary remains behind in the slurry. The object of flotation is to separate and recover as much of the valuable constituent(s) as possible 20 in as high a concentration as possible, which is then made available for further downstream processing steps.

In certain embodiments, the composition serves as a collector and/or a frother. In certain embodiments, the composition serves as a performance enhancer or a modifier for traditional collectors and/or frothers.

25 In certain embodiments, the composition further comprises a chemical collector or frother in combination with the biosurfactant or biosurfactant-producing microbial culture. Other additional additives can include, for example, promoters, regulators, modifiers, depressors (deactivators) and/or activators, which enhance the selectivity of the flotation step and facilitate the removal of the concentrate from the slurry.

30 In preferred embodiments, the subject invention provides methods for separating and/or extracting particles of a target metal or mineral (e.g., the beneficiary) from a particulate source of said target metal, wherein a beneficiation composition of the subject invention is applied to the particles of the source in a froth flotation medium, or slurry. In certain embodiments, the source is coal or a coal combustion waste product, such as, for example, coal fly ash. In certain embodiments,

the source is rock, ore, or tailings sourced from a mine or quarry, including, for example, from iron bearing minerals, such as, for example, hematite, goethite, magnetite, martite, or limonite.

In certain embodiments, prior to froth flotation, the methods comprise subjecting the source (e.g., ore, tailings, rock, or fly ash) to roughing or comminution. Roughing or comminution reduces the size and/or size distribution of the source particles and, in some embodiments, liberates some of the target mineral or metal from gangue materials. This can be achieved via, for example, milling, micronizing, pulverizing or otherwise grinding the source particles to particles and/or fines having a desired size. In some embodiments, the desired particle size is between 10 μm and 5 mm. In certain embodiments, “fines” have a particle size less than about 3.5 mm. In certain embodiments, the methods enable larger particles to be floated relative to source particles that have not been treated with the subject compositions, thereby reducing greenhouse gas emissions produced to decrease particle size by, for example, grinding a mined material.

In certain embodiments, the method comprises applying the source particles and/or fines to water to form a slurry and aerating the slurry in the presence of a beneficiation composition according to the subject invention. In certain embodiments, the composition serves as a collector to facilitate the attachment of air bubbles to particles of the target metal or mineral (e.g., the concentrate), which allows for flotation of the concentrate particles to the surface of the liquid slurry. In alternative embodiments, the composition serves as a collector to facilitate the attachment of air bubbles to particles of gangue, which allows for flotation of the gangue particles to the surface of the liquid slurry. In some embodiments, the method is performed in a tank, vat, column, pool or other vessel.

In certain embodiments, the beneficiation composition serves as a frother to reduce the surface tension of the liquid slurry. In some embodiments, this promotes the even distribution of air bubbles throughout the slurry to enhance adsorption of the concentrate or gangue particles thereto. In some embodiments, the reduced surface tension promotes the formation of a stable froth layer at the surface of the slurry. In some embodiments, a stable froth layer prevents the air bubbles from breaking and dropping the adsorbed concentrate or gangue particles back into the slurry. In certain embodiments, the surface froth layer comprises the concentrate or gangue particles, which can then flow from, or be mechanically skimmed from, the slurry, and collected.

In certain embodiments, the concentrate or gangue particles are detached from the froth bubbles after collection of the froth. The particles can be washed, dried, incinerated, and/or processed by any other means known in the metallurgical arts. Detachment of the particles can be achieved by, for example, applying shear force, such as through the use of an impeller or other mechanical mixer. In certain embodiments, the separated concentrate or gangue particles are dried using, for example, a rotary dryer, a convection dryer, a conveyer, or a fluidized-bed dryer.

The leftover gangue or concentrate materials remaining in the slurry, or tailings, can be collected and re-treated according to the subject methods, if desired, or treated using other beneficiation systems to produce, e.g., concrete-grade ash, copper concentrate, zinc concentrate, iron concentrate, and nickel concentrate.

5 The biosurfactant-based beneficiation composition can be applied with a traditional chemical collector or frother as a modifier, an adjuvant and/or enhancer for the collector or frother. Advantageously, in certain embodiments, the biosurfactant(s) of the subject composition work in synergy with the collectors and/or frothers to improve their performance. This not only allows for potentially reduced volume usage of harsh chemical reagents, but also enhanced separation yields
10 with reduced time and/or energy expenditure, which can in turn improve the environmental impact of the process, particularly reducing greenhouse gas emissions.

 Thus, in certain embodiments, the subject invention provides methods of enhancing the performance of standard collectors or frothers, wherein the reagents are applied to the froth flotation slurry alongside a biosurfactant and/or a biosurfactant-producing microbial culture according to the
15 subject invention.

 In certain embodiments, the target metal or mineral that is recovered from the rock, ore, tailings, and/or coal combustion waste is a valuable compound, such as gold, silver, platinum group metals (platinum, palladium, rhodium, ruthenium, osmium and iridium), and/or rare earth metals (lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, and lutetium). In certain embodiments,
20 the concentration of the target metal or mineral in the concentrate is at least 2% by weight. In certain embodiments, a coal combustion waste product can serve as the source of the target mineral or metal, including, for example, fly ash, bottom ash or boiler slag obtained from a coal combustion plant, wherein the coal is anthracite, bituminous, subbituminous and/or lignite coal.

25 In certain embodiments, the rock, ore, or tailings that serve as the source of the target mineral or metal is obtained from a mine or quarry. In certain embodiments, the mine can be an iron ore mine, copper mine, copper-nickel mine, tin mine, nickel mine, gold mine, silver mine, molybdenum mine, aluminum mine, lead-zinc mine, tungsten mine, zinc mine, ruthenium mine, palladium mine, osmium mine, iridium mine, osmiridium mine, or platinum mine. In certain embodiments, the
30 palladium or nickel mine can be a source of rhodium. The mine can be an underground mine, surface mine, placer mine or in situ mine. In certain embodiments, the quarry can extract chalk, clay, cinder, coal, sand, gravel, coquina, diabase, gabbro, granite, gritstone, gypsum, limestone, marble, ores, phosphate rock, quartz, sandstone, slate, travertine, or any combination thereof.

 In certain embodiments, the biosurfactant of the subject compositions is utilized in crude
35 form. The crude form can comprise, in addition to the biosurfactant, fermentation broth in which a

biosurfactant-producing microorganism was cultivated, residual microbial cell matter or live or inactive microbial cells, residual nutrients, and/or other microbial growth by-products.

In some embodiments, the biosurfactant is utilized after being extracted from a fermentation broth and, optionally, purified.

5 The biosurfactant according to the subject invention can be, for example, a glycolipid (e.g., sophorolipids, rhamnolipids, cellobiose lipids, mannosylerythritol lipids and trehalose lipids), lipopeptide (e.g., surfactin, iturin, fengycin, arthrofatin, and lichenysin), flavolipid, phospholipid (e.g., cardiolipins), fatty acid ester compound, fatty acid ether compound, and/or high molecular weight polymers such as lipoproteins, lipopolysaccharide-protein complexes, and polysaccharide-
10 protein-fatty acid complexes.

In certain specific embodiments, the biosurfactant is a sophorolipid (SLP), including linear SLP, lactonic SLP, acetylated SLP, de-acetylated SLP, salt-form SLP, esterified SLP derivatives, amino acid-SLP conjugates, and other SLP derivatives or isomers that exist in nature and/or are produced synthetically. In preferred embodiments, the SLP is a linear SLP or a derivatized linear
15 SLP. In certain embodiments, the subject compositions can comprise lactonic and linear SLP, with at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the SLP comprising linear forms, and the remainder comprising, for example, lactonic forms.

Advantageously, the methods of the subject invention do not require complicated equipment
20 or high energy consumption, can reduce greenhouse gas emissions, and production of the composition can be performed on site, for example, at a mine or refinery.

DETAILED DESCRIPTION OF THE INVENTION

The subject invention provides compositions and methods for reducing atmospheric carbon
25 and/or other greenhouse gas emissions using additives in mining and/or refining processes. In preferred embodiments, a composition comprising one or more microorganisms and/or one or more microbial growth by-products is contacted with an ore, gangue, or other substance containing a substance of interest, such as, for example, a metal, mineral, or element. The composition is capable of, for example, reducing the use of fossil fuels, and thus, reducing the amount of carbon emissions
30 produced from mining and/or subsequent transportation or refining processes.

Selected Definitions

As used herein, “applying” a composition or product refers to contacting it with a target or site such that the composition or product can have an effect on that target or site. The effect can be

due to, for example, microbial growth and/or the action of a biosurfactant or other microbial growth by-product.

As used herein, a “biofilm” is a complex aggregate of microorganisms, such as bacteria, yeast, or fungi, wherein the cells adhere to each other and/or to a surface using an extracellular matrix. The cells in biofilms are physiologically distinct from planktonic cells of the same organism, which are single cells that can float or swim in liquid medium.

As used herein, an “isolated” or “purified” nucleic acid molecule, polynucleotide, polypeptide, protein or organic compound such as a small molecule (e.g., those described below), is substantially free of other compounds, such as cellular material, with which it is associated in nature. A purified or isolated polynucleotide (ribonucleic acid (RNA) or deoxyribonucleic acid (DNA)) is free of the genes or sequences that flank it in its naturally-occurring state. A purified or isolated polypeptide is free of the amino acids or sequences that flank it in its naturally-occurring state. An isolated microbial strain means that the strain is removed from the environment in which it exists in nature. Thus, the isolated strain may exist as, for example, a biologically pure culture, or as spores (or other forms of the strain) in association with a carrier.

In certain embodiments, purified compounds are at least 60% by weight the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 98%, by weight the compound of interest. For example, a purified compound is one that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 98%, 99%, or 100% (w/w) of the desired compound by weight. Purity is measured by any appropriate standard method, for example, by column chromatography, thin layer chromatography, or high-performance liquid chromatography (HPLC) analysis.

A “metabolite” refers to any substance produced by metabolism or a substance necessary for taking part in a particular metabolic process. A metabolite can be an organic compound that is a starting material, an intermediate in, or an end product of metabolism. Examples of metabolites include, but are not limited to, enzymes, acids, solvents, alcohols, proteins, vitamins, minerals, microelements, amino acids, biopolymers and biosurfactants.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 20 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20, as well as all intervening decimal values between the aforementioned integers such as, for example, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9. With respect to sub-ranges, “nested sub-ranges” that extend from either end point of the range are specifically contemplated. For example, a nested sub-range of an exemplary range of 1 to 50 may comprise 1 to 10, 1 to 20, 1 to 30, and 1 to 40 in one direction, or 50 to 40, 50 to 30, 50 to 20, and 50 to 10 in the other direction.

As used herein a “reduction” means a negative alteration, and an “increase” means a positive alteration, wherein the negative or positive alteration is at least 0.001%, 0.01%, 0.1%, 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

5 As used herein, “surfactant” means a compound that lowers the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants act as, e.g., detergents, wetting agents, emulsifiers, foaming agents, and/or dispersants. A “biosurfactant” is a surface-active substance produced by a living cell and/or using naturally-derived substrates.

10 Biosurfactants are a structurally diverse group of surface-active substances consisting of two parts: a polar (hydrophilic) moiety and non-polar (hydrophobic) group. Due to their amphiphilic structure, biosurfactants can, for example, increase the surface area of hydrophobic water-insoluble substances, increase the water bioavailability of such substances, and change the properties of bacterial cell surfaces. Biosurfactants can also reduce the interfacial tension between water and oil and, therefore, lower the hydrostatic pressure required to move entrapped liquid to overcome the
15 capillary effect. Biosurfactants accumulate at interfaces, thus reducing interfacial tension and leading to the formation of aggregated micellar structures in solution. The formation of micelles provides a physical mechanism to mobilize, for example, oil in a moving aqueous phase.

The ability of biosurfactants to reduce the surface tension also permits their use as antibacterial, antifungal, and hemolytic agents to, for example, control pests and/or microbial
20 growth.

Typically, the hydrophilic group of a biosurfactant is a sugar (e.g., a mono-, di-, or polysaccharide) or a peptide, while the hydrophobic group is typically a fatty acid. Thus, there are countless potential variations of biosurfactant molecules based on, for example, type of sugar, number of sugars, size of peptides, which amino acids are present in the peptides, fatty acid length,
25 saturation of fatty acids, additional acetylation, additional functional groups, esterification, polarity and charge of the molecule.

These variations lead to a group of molecules comprising a wide variety of classes, including, for example, glycolipids (e.g., sphorolipids, rhamnolipids, cellobiose lipids, mannosylerythritol lipids and trehalose lipids), lipopeptides (e.g., surfactin, iturin, fengycin,
30 arthrofatin and lichenysin), flavolipids, phospholipids (e.g., cardiolipins), fatty acid ester compounds, and high molecular weight polymers such as lipoproteins, lipopolysaccharide-protein complexes, and polysaccharide-protein-fatty acid complexes. Each type of biosurfactant within each class can further comprise subtypes having further modified structures.

Like chemical surfactants, each biosurfactant molecule has its own HLB value depending
35 on its structure; however, unlike production of chemical surfactants, which results in a single

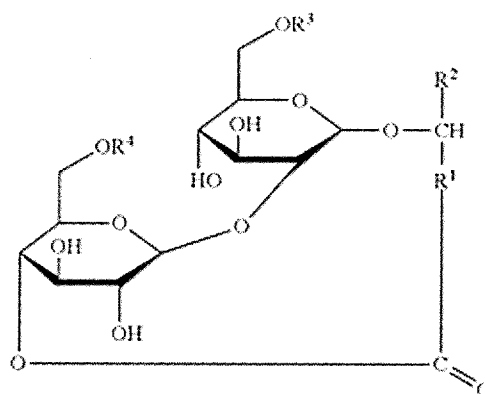
molecule with a single HLB value or range, one cycle of biosurfactant production typically results in a mixture of biosurfactant molecules (e.g., subtypes and isomers thereof).

The phrases “biosurfactant” and “biosurfactant molecule” include all forms, analogs, orthologs, isomers, and natural and/or anthropogenic modifications of any biosurfactant class (e.g., glycolipid) and/or subtype thereof (e.g., sophorolipid).

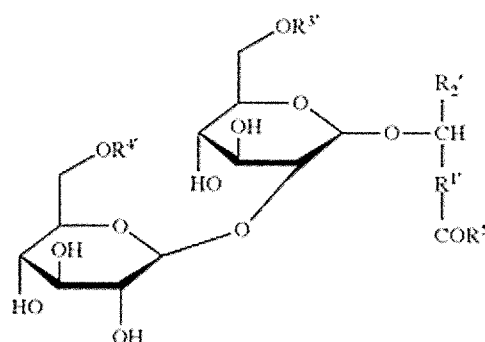
As used herein, the term “sophorolipid,” “sophorolipid molecule,” “SLP” or “SLP molecule” includes all forms, and isomers thereof, of SLP molecules, including, for example, acidic (linear) SLP (ASL) and lactonic SLP (LSL). Further included are mono-acetylated SLP, di-acetylated SLP, esterified SLP, SLP with varying hydrophobic chain lengths, cationic and/or anionic SLP with fatty acid-amino acid complexes attached, esterified SLP, SLP-metal complexes, SLP-salt derivatives (e.g., a sodium salt of a linear SLP), and other, including those that are and/or are not described within in this disclosure.

In some embodiments, the SLP molecules according to the subject invention are represented by General Formula (1) and/or General Formula (2) and are obtained as a collection of multiple types of structural homologues:

(1)



(2)



where R^1 and $R^{1'}$ independently represent saturated hydrocarbon chains or single or multiple, in particular single, unsaturated hydrocarbon chains having 8 to 20 carbon atoms, which can be

linear or branched and can comprise one or more hydroxy groups, R^2 and R^2 independently represent a hydrogen atom or a saturated alkyl functional group or a single or multiple, in particular single, unsaturated alkyl functional group having 1 to 9 carbon atoms, more preferably 1 to 4 carbon atoms, which can be linear or branched and can comprise one or more hydroxy groups, and R^3 , R^3 , R^4 and R^4 independently represent a hydrogen atom or $-\text{COCH}_3$. R^5 is typically, but not limited to, $-\text{OH}$.

SLP are typically produced by yeasts, such as *Starmerella* spp. yeasts and/or *Candida* spp. yeasts, e.g., *Starmerella (Candida) bombicola*, *Candida apicola*, *Candida batistae*, *Candida floricola*, *Candida riidocensis*, *Candida stellate* and/or *Candida kuoi*. SLP have environmental compatibility, high biodegradability, low toxicity, high selectivity and specific activity in a broad range of temperature, pH and salinity conditions. Additionally, in some embodiments, SLP can be advantageous due to their small micelle size, which can help facilitate the movement of the micelle, and compounds enclosed therein, through nanoscale pores and spaces. In certain embodiments, the micelle size of a SLP is less than 100 nm, less than 50 nm, less than 20 nm, less than 15 nm, less than 10 nm, or less than 5 nm.

As used herein, “beneficiation” refers to the process by which gangue materials are removed from the product of interest (e.g., element, compound, or mineral).

As used herein, “ore” refers to a naturally occurring solid material from which a valuable substance, mineral and/or metal can be profitably extracted. Ores are often mined from ore deposits, which comprise ore minerals containing the valuable substance. “Gangue” minerals are minerals that occur in the deposit but do not contain the valuable substance. Examples of ore deposits include hydrothermal deposits, magmatic deposits, laterite deposits, volcanogenic deposits, metamorphically reworked deposits, carbonatite-alkaline igneous related deposits, placer ore deposits, residual ore deposits, sedimentary deposits, sedimentary hydrothermal deposits and astrobleme-related deposits. Ores, as defined herein, however, can also include ore concentrates or tailings.

As used herein, “leaching” refers to the process by which metal is extracted from ore by solutions including by, for example, ammonia leaching, alkali leaching, acid leaching, cyanidation (i.e., cyanide leaching), or thiosulfate leaching. As used herein “cyanidation” refers to the process of converting gold in ore to a water-soluble coordination complex using aqueous cyanide, including, for example, sodium cyanide, potassium cyanide, or calcium cyanide.

As used herein, “source” in the context of a source of a target metal or mineral refers to a material from which a valuable substance, mineral and/or metal can be profitably extracted. Sources can include ores, which are often mined from ore deposits with minerals containing the target mineral or metal. Other “gangue” minerals are minerals containing non-target metals that occur in the source. Examples of ore deposits include hydrothermal deposits, magmatic deposits, laterite

deposits, volcanogenic deposits, metamorphically reworked deposits, carbonatite-alkaline igneous related deposits, placer ore deposits, residual ore deposits, sedimentary deposits, sedimentary hydrothermal deposits and astrobleme-related deposits. Sources, as used herein, however, can also include rocks, ore concentrates or tailings, coal or coal combustion waste products, or even other sources of metal or valuable minerals, including but not limited to, jewelry, electronic scraps, batteries and other scrap materials.

As used herein, “slurry” means a mixture comprising a liquid medium within which particles and/or fines of concentrate and tailings are dispersed or suspended, e.g., during froth flotation. The liquid medium may be water, partially water, or may not contain any water at all, and/or can include alcohol, aromatic liquid, phenol, azeotropes, and any combination thereof.

As used herein, “collector” means a composition that selectively adheres to a specific type of particulate substance present in a froth flotation slurry and facilitates the adhesion of the particulate substance to air bubbles that result from aeration of the slurry. In certain embodiments, the particulate substance is hydrophobic in nature. In certain embodiments, the collector increases the hydrophobicity of the particulate substance. Examples of collectors include oily products such as fuel oil, tar oil, animal oil, vegetable oil, fatty acids, fatty acid esters, fatty amines, hydrophobic polymers, neutralized fatty acids, soaps, amine compounds, petroleum-based oily compounds (such as diesel fuels, decant oils, and light cycle oils, kerosene or fuel oils), an organic collector (e.g., xanthates, xanthogen formates, thionocarbamates, dithiophosphates (including sodium, zinc and other salts of dithiophosphates), and mercaptans (including mercaptobenzothiazole), ethyl octylsulfide), and any combination thereof.

As used herein, a “concentrate” means a portion of a source that is separated from a slurry by flotation and collected within the froth layer. By contrast, “tailings” mean a portion of the source that remains in the slurry.

As used herein, a “frother” or “frothing agent” means a composition that reduces the surface tension of liquids, enhance the formation of air bubbles and/or preserve formed air bubbles that are produced by aeration of a froth flotation slurry. Examples of frothers include but are not limited to aliphatic alcohols, cyclic alcohols, propylene oxide and polypropylene oxide, propylene glycol, polypropylene glycol and polypropylene glycol ethers, polyglycol ethers, polyglycol glycerol ethers, polyoxyparaffins, natural oils such as pine oil an alcohol blend which is from the waste stream of the production of 2-ethyl hexanol and any combination thereof.

As used herein, “leaching” refers to the process by which a metal is separated from a metal source, such as, for example, ore, mine tailings, slag and/or electronic waste from, for example, batteries and circuits by aqueous solutions including by, for example, cyanide leaching, ammonia leaching, alkali leaching, or acid leaching.

The transitional term “comprising,” which is synonymous with “including,” or “containing,” is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase “consisting of” excludes any element, step, or ingredient not specified in the claim. The transitional phrase “consisting essentially of” limits the scope of a claim to the specified materials or steps “and those that do not materially affect the basic and novel characteristic(s)” of the claimed invention. Use of the term “comprising” contemplates other embodiments that “consist” or “consist essentially of” the recited component(s).

Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms “a,” “and” and “the” are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All references cited herein are hereby incorporated by reference in their entirety.

Beneficiation Composition to Reduce Greenhouse Gas Emissions

In certain preferred embodiments, the subject invention provides a beneficiation composition for use in metal and mineral beneficiation, including, for example, in methods of grinding, leaching, and froth flotation, including, for example, direct flotation or reverse flotation.

In certain embodiments, the beneficiation composition comprises a microbe-based product comprising a biosurfactant in crude form. The crude form can comprise, in addition to the biosurfactant, fermentation broth in which a biosurfactant-producing microorganism was cultivated, residual microbial cell matter or live or inactive microbial cells, residual nutrients, and/or other microbial growth by-products. The product may be, for example, at least, by weight, 1%, 5%, 10%, 25%, 50%, 75%, or 100% broth. The amount of biomass in the product, by weight, may be, for example, anywhere from 0% to 100% inclusive of all percentages therebetween.

In some embodiments, the biosurfactant is utilized after being extracted from a fermentation broth and, optionally, purified.

In some embodiments, the beneficiation composition comprises an aqueous solution of a biosurfactant or a blend of biosurfactants. The biosurfactant can be present in the solution from, for example, 1.0 % to more than 10% of the composition, or from 0.1% to more than 25% of the composition.

5 In some embodiments, the biosurfactant can be included in the composition at 0.01 to 99.9%, 0.1 to 90%, 0.5 to 80%, 0.75 to 70%, 1.0 to 50%, 1.5 to 25%, or 2.0 to 15% by weight, with respect to the total beneficiation composition.

In some embodiments, the biosurfactant can be included in the composition at, for example, 0.01 to 100,000 ppm, 0.05 to 10,000 ppm, 0.1 to 1,000 ppm, 0.5 to 750 ppm, 1.0 to 500 ppm, 2.0 to
10 250 ppm, or 3.0 to 100 ppm, with respect to the amount of source being treated.

In another embodiment, purified biosurfactants may be added in combination with an acceptable carrier, in that the biosurfactant may be presented at concentrations of 0.001 to 50% (v/v), preferably, 0.01 to 20% (v/v), more preferably, 0.02 to 5% (v/v).

In certain specific embodiments, the composition comprises a mixture of ASL and one or
15 more other SLP molecules, such as, for example, LSL. In one embodiment, the percentage of ASL in the composition is about 20% to 50%, or 25% to 30% (with 100% being the sum of the amount of SLP molecules). In one embodiment, the percentage of ASL is at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or greater with respect to the total sum of SLP molecules. In other embodiments, the percentage of
20 ASL is over 75%, over 90%, or over 99% with respect to the total sum of SLP molecules.

In order to achieve higher ratios of ASL over the LSL and/or other SLP molecules, the mixture can be subjected to purification via, for example, solvent extraction, alkaline hydrolysis, water washing, oil washing and/or as described in International Patent Publication WO 2021/127339 A1, incorporated herein by reference.

25 In some embodiments, the composition comprises further components such as cellular matter, broth components, residual feedstock materials (e.g., fatty acids and glucose), and/or additional metabolites from the SLP-producing microorganism that are present at a concentration of, for example, from 50% to 0.001%, from 40% to 1%, or from 25% to 5%, of the total composition volume. In certain embodiments, one or more of these additional components contributes to
30 enhanced activity of the bioleaching composition, compared with the activity of bioleaching compositions comprising higher purity biosurfactant mixtures containing, for example, less than 15%, less than 10%, less than 5%, or less than 1% non-SLP materials and/or components.

In some embodiments, the composition comprises more than one type of biosurfactant, for example, a glycolipid and another glycolipid, or a glycolipid and a lipopeptide.

In certain embodiments, the composition further comprises a chemical collector or frother in combination with the biosurfactant or biosurfactant-producing microbial culture. Other additional additives can include, for example, promoters, regulators, modifiers, depressors (deactivators) and/or activators, which enhance the selectivity of the flotation step and facilitate the removal of the concentrate from the slurry; and/or carriers, other microbe-based compositions, additional biosurfactants or chemical surfactants, leaching reagents (e.g., cyanide, acids, oxidants, and microbial leaching agents), and other ingredients specific for an intended use.

In certain embodiments, the composition comprises one or more metal solubilizing agents and an adjuvant, or performance booster, for the one or more metal solubilizing agents, wherein the adjuvant is a biosurfactant-producing microorganism and/or a biosurfactant.

In certain embodiments, the composition comprises a biosurfactant and one or more metal solubilizing agents, wherein the metal solubilizing agent is an acid, an oxidant, an acid-oxidant mixture, or a microbial leaching agent, wherein the biosurfactant serves as an acid leaching accelerant, a bio-oxidation accelerant, and/or a bioleaching accelerant.

In certain embodiments, the subject invention provides pre-leaching compositions comprising components that are derived from microorganisms. In certain embodiments, the pre-leaching composition comprises a microbial biosurfactant. In certain embodiments, the composition comprises a biosurfactant, and, optionally, one or more of chemical surfactants, pine oils, xanthates, or any combination thereof.

In certain embodiments, the chemical surfactant of the composition is a detergent, wetting agent, emulsifier, foaming agent, and/or dispersant. In some embodiments, the chemical surfactant can be included in the pre-leaching composition at 0.01 to 99.9%, 0.1 to 90%, 0.5 to 80%, 0.75 to 70%, 1.0 to 50%, 1.5 to 25%, or 2.0 to 15% by weight, with respect to the total composition.

In certain embodiments, the pine oil of the composition comprises α -terpineol, terpene alcohols, terpene hydrocarbons, terpene ethers, terpene esters, or any combination thereof. In some embodiments, the pine oil can be included in the composition at 0.01 to 99.9%, 0.1 to 90%, 0.5 to 80%, 0.75 to 70%, 1.0 to 50%, 1.5 to 25%, or 2.0 to 15% by weight, with respect to the total composition.

In certain embodiments, the xanthate of the composition is sodium ethyl xanthate, potassium ethyl xanthate, sodium isopropyl xanthate, sodium isobutyl xanthate, potassium amyl xanthate, or any combination thereof. In some embodiments, the xanthate can be included in the composition at 0.01 to 99.9%, 0.1 to 90%, 0.5 to 80%, 0.75 to 70%, 1.0 to 50%, 1.5 to 25%, or 2.0 to 15% by weight, with respect to the total composition.

In certain embodiments, the composition comprises an acid. In some embodiments, the acid is an organic acid, such as, for example, acetic acid, citric acid, lactic acid, butyric acid, sorbic acid,

benzoic acid, formic acid, fumaric acid, propionic acid, ascorbic acid, glyoxylic acid, malonic acid, pyruvic acid, oxalic acid, uric acid, malic acid, tartaric acid and/or analogs thereof. In certain embodiments, the acid is selected from inorganic acids such as, for example, sulfuric acid, nitric acid, phosphoric acid, hydrochloric acid, hydrofluoric acid, boric acid and analogs thereof. In
5 preferred embodiments, the acid is a strong acid, such as sulfuric acid.

In some embodiments, the acid can be included in the composition at 0.01 to 99.9%, 0.1 to 90%, 0.5 to 80%, 0.75 to 70%, 1.0 to 50%, 1.5 to 25%, or 2.0 to 15% by weight, with respect to the total composition.

In certain embodiments, the composition comprises an oxidant. In some embodiments, the
10 oxidant is, for example, ferric chloride, chlorine, bromine, or oxygen. The oxidant can further be selected from, for example, a ferric salt, commonly ferric sulfate, or a ferric salt with an anion equivalent of the acid in full dissociated form.

In some embodiments, the oxidant can be included in the composition at 0.01 to 99.9%, 0.1 to 90%, 0.5 to 80%, 0.75 to 70%, 1.0 to 50%, 1.5 to 25%, 2.0 to 15%, or less than about 7.0% by
15 weight, with respect to the total composition.

The composition can further comprise other additives such as, for example, carriers, other microbe-based compositions, additional biosurfactants, biocides, enzymes, catalysts, pH adjusting agents, solvents, salts (including inorganic salts), buffers, filtering aids, clarifying agents, flocculants, chelating agents, acids, emulsifying agents, lubricants, solubility controlling agents,
20 preservatives, stabilizers, ultra-violet light resistant agents, viscosity modifiers, preservatives, tracking agents, and other microbes and other ingredients specific for an intended use.

In certain embodiments, chelating agents can be, but are not limited to, ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), a phosphonate, succimer (DMSA), diethylenetriaminepentaacetate (DTPA), *N*-acetylcysteine, *n*-
25 hydroxyethylethylenediaminetriacetic acid (HEDTA), organic acids with more than one coordination group (e.g., rubeanic acid), STPP (sodiumtripolyphosphate, Na₅P₃O₁₀), trisodium phosphate (TSP), water, carbohydrates, organic acids with more than one coordination group (e.g., citric acid), lipids, steroids, amino acids or related compounds (e.g., glutathione), peptides, phosphates, nucleotides, tetrapyrrols, ferrioxamines, ionophores, orphenolics, sodium citrate,
30 sodium gluconate, ethylenediamine disuccinic acid (EDDS), iminodisuccinic acid (IDS), L-glutamic acid diacetic Acid (GLDA), GLDA-Na₄, methyl glycindiacytic acid (MGDA), polyaspartic acid (PASA), hemoglobin, chlorophyll, lipophilic β -diketone, and (14,16)-hentriacontanedione, ethylenediamine-N,N'-diglutaric acid (EDDG), ethylenediamine-N,N'-dimalonic acid (EDDM), 3-hydroxy-2,2-iminodisuccinic acid (HIDS), 2-hydroxyethyliminodiacetic acid (HEIDA), pyridine-
35 2,6-dicarboxylic acid (PDA), trimethyl glycine (TMG), Tiron, or any combination thereof.

In certain embodiments, the composition can be a liquid, a solid or a semi-solid, such as, for example, a powder or a paste.

Target Substances

5 In certain embodiments, the substances that can be contacted to the subject compositions can be derived from a mining or quarrying site that produces, for example, cobalt (e.g., erythrite, skytterudite, cobaltite, carrollite, linnaeite, and asbolite (asbolane)); copper (e.g., chalcopyrite, chalcocite, bornite, djurleite, malachite, azurite, chrysocolla, cuprite, tenorite, native copper and brochantite); gold (e.g., native gold, electrum, tellurides, calaverite, sylvanite and petzite); silver
10 (e.g., sulfide argentite, sulfide acanthite, native silver, sulfosalts, pyrargyrite, proustite, cerargyrite, tetrahedrites); aluminum (e.g., bauxite, gibbsite, bohmeite, diaspore); antimony (e.g., stibnite); barium (e.g., barite, witherite); cesium (e.g., pollucite); chromium (e.g., chromite); iron (e.g., hematite, magnetite, pyrite, pyrrhotite, goethite, siderite); lead (e.g., galena, cerussite, anglesite); lithium (e.g., pegmatite, spodumene, lepidolite, petalite, amblygonite, lithium carbonate);
15 magnesium (e.g., dolomite, magnesite, brucite, carnallite, olivine); manganese (e.g., hausmannite, pyrolusite, barunite, manganite, rhodochrosite); mercury (e.g., cinnabar); molybdenum (e.g., molybdenite); nickel (e.g., pentlandite, pyrrhotite, garnierite); phosphorus (e.g., hydroxylapatite, fluorapatite, chlorapatite); platinum group (platinum, osmium, rhodium, ruthenium, palladium) (e.g., native elements or alloys of platinum group members, sperrylite); potassium (e.g., sylvite,
20 langbeinite); rare earth elements (cerium, dysprosium, erbium, europium, gadolinium, holmium, lanthanum, lutetium, neodymium, praseodymium, samarium, scandium, terbium, thulium, ytterbium, yttrium) (e.g., bastnasite, monazite, loparite); sodium (e.g., halite, soda ash); strontium (e.g., celestite, strontianite); sulfur (e.g., native sulfur, pyrite); tin (e.g., cassiterite); titanium (e.g., scheelite, huebnerite-ferberite); uranium (e.g., uraninite, pitchblende, coffinite, carnotite, autunite);
25 vanadium; zinc (e.g., sphalerite, zinc sulfide, smithsonite, hemimorphite); and/or zirconium (e.g., zircon).

In preferred embodiments, the mining site can be a coal mine, iron ore mine, copper mine, copper-nickel mine, tin mine, nickel mine, gold mine, silver mine, molybdenum mine, aluminum mine (e.g., bauxite mine, kyanite mine), lead-zinc mine, tungsten mine, or zinc mine. The mine can
30 be, for example, an underground mine, surface mine, placer mine or in situ mine.

Additional elements and/or minerals that the particle may include are, for example, arsenic, bertrandite, bismuthinite, borax, colemanite, kernite, ulexite, sphalerite, halite, gallium, germanium, hafnium, indium, iodine, columbite, tantalite-columbite, rubidium, quartz, diamonds, garnets (almandine, pyrope and andradite), corundum, barite, calcite, clays, feldspars (e.g., orthoclase,
35 microcline, albite); gemstones (e.g., diamonds, rubies, sapphires, emeralds, aquamarine, kunzite);

gypsum; perlite; sodium carbonate; zeolites; chabazite; clinoptilolite; mordenite; wollastonite; vermiculite; talc; pyrophyllite; graphite; kyanite; andalusite; muscovite; phlogopite; menatite; magnetite; dolomite; ilmenite; wolframite; beryllium; tellurium; bismuth; technetium; potash; rock salt; sodium chloride; sodium sulfate; nahcolite; niobium; tantalum and any combination of such substances or compounds containing such substances.

Pre-leaching Methods with Reduced Greenhouse Gas Emissions

In certain embodiments, the subject invention provides a method for extracting impurities from a metal source with reduced greenhouse gas emissions, particularly carbon dioxide. In certain embodiments, the subject invention provides a method for removing impurities from a metal source, wherein the method comprises the step of: (i) obtaining the metal source, said metal source comprising a metal and an impurity; (ii) contacting a pre-leaching composition according to the subject invention with the metal source for a period of time to yield a mixture comprising a treated metal source and an impurity; and (iii) separating the impurity and pre-leaching composition with the metal from the mixture. The method can be carried out, *in situ*, in a heap leach pad, a column, or any other laboratory or industrial sized reactor.

In some embodiments, step (i) comprises grinding the obtained metal source into a fine powder. The particles in the powder can be less than about 1 m, about 75 cm, about 50 cm, about 25 cm, about 10 cm, about 1 cm about 75 mm, about 50 mm, about 25 mm, about 10 mm, about 5 mm, about 1 mm, about 100 μ m, about 10 μ m, about 1 μ m, about 100 nm, about 10 nm, about 1 nm in diameter. In certain embodiments, the subject compositions and methods can decrease greenhouse gas emissions during the leaching process by, for example, reducing the amount of grinding necessary during refining, thereby reducing the amount of energy consumed during the grinding process. In certain embodiments, the particles in the powder can be at least about 1 cm, 10 cm, about 25 cm, about 50 cm, about 75 cm, or about 1 m in diameter.

In some embodiments, step (ii) comprises applying a pre-leaching composition comprising a biosurfactant and, optionally, water, other surfactants, pine oils, and xanthates, to the metal source. In certain embodiments, air can be pumped into the mixture and the metal and biosurfactants can rise to the top of the mixture, creating a froth. In some embodiments, the froth can be removed and tailings or other non-metal containing components can be removed from the mixture. Step (ii) can be repeated as many times as necessary to achieve a desired reduction in impurity content. In certain embodiments, the subject compositions and methods can decrease greenhouse gas emissions during the leaching processes by, for example, increasing the efficiency of the leaching process by increasing the rate of leaching or concentration of a target substance in a leachate, which is liquid that has percolated through a solid and leached out some of the substances of interest (e.g., metals

or minerals), relative to a leachate that has not been contacted to a composition of the subject invention, thereby reducing the energy needed to transport or pump the leachate.

In certain embodiments, the time period of step (ii) is from 1 minute to 48 hours, about 30 minutes to 40 hours, or preferably about 12 hours to 24 hours. In certain embodiments, step (ii) comprises applying a liquid form pre-leaching composition to the metal source to produce a liquid mixture and stirring or otherwise agitating the liquid mixture for the period of time. In certain
5 embodiments, the subject compositions and methods can decrease greenhouse gas emissions during the leaching processes by, for example, increasing the efficiency of the leaching process by decreasing the time needed for leaching relative to a leaching process that has not been contacted to
10 a composition of the subject invention, thereby reducing the energy needed to transport or pump a leaching composition or a leachate.

In some embodiments, when step (ii) is carried out in liquid, the impurity is present in the aqueous phase and the metal floats in a froth above the aqueous phase that can be removed from the liquid. In certain embodiments, the subject compositions and methods can decrease greenhouse gas
15 emissions during the leaching processes by, for example, increasing the efficiency of the leaching process by increasing the efficiency of flotation, in which a higher concentration of a substance of interest is in the froth relative to a froth that has not been contacted to a composition of the subject invention, thereby reducing the energy needed to transport or pump froth.

In some embodiments, step (iii) comprises applying a leaching solution and optionally,
20 mixing under agitation (e.g., shaking or stirring) for a period of time (e.g., 10 hours to 48 hours). The leaching solution will comprise the impurity, pre-leaching composition, and the metal that is to be removed from the fluid.

In certain embodiments, the method comprises (a) obtaining the metal source, said metal source comprising a metal and an impurity; (b) applying a pre-leaching composition according to
25 the subject invention to the metal source under agitation for a period of time to yield a mixture comprising a treated metal source and an impurity; (c) preparing a slurry of the metal source in water and maintaining the slurry under agitation and air bubbling, thereby causing the formation of a froth comprising the treated metal source and an aqueous layer comprising impurities; and (e) separating the treated metal from the aqueous layer.

In some embodiments, step (e) comprises applying a leaching solution to the mixture, optionally under agitation (e.g., shaking or stirring) for a period of time (e.g., about 10 hours to about
30 48 hours). The leaching solution will comprise the impurity, pre-leaching composition, and the metal that is to be removed from the fluid.

The methods of the subject invention can be carried out at ambient temperature, and/or at a
35 temperature of about 15°C to about 50°C, about 20°C to about 40°C, about 20°C to about 35°C,

about 20°C to about 30° C, about 25° C, about 40°C to 120°C, about 50°C to about 100°C, about 60°C to about 100°C, about 70°C to about 100°C, about 80°C to about 100°C, or about 100°C. In certain embodiments, a temperature higher than ambient temperature can be provided using, for example, a microwave, ultrasound, induction heating, plasma, electricity, or any combination thereof.

The methods of the subject invention can be carried out at ambient pressure, and/or at a pressure of about 50 bars, 75 bar, 100 bars, or greater than 100 bars.

In certain embodiments, the amount of the pre-leaching composition applied is about 0.1 to 15%, about 0.1 to 10%, about 0.1 to 5%, about 0.1 to 3%, about 0.1%, or about 1 vol % based on an amount of the metal-containing material.

In certain embodiments, the methods of the subject invention result in at least 25% reduction in impurities content, preferably at least 50% reduction, after one treatment. In some embodiments, the metal source can be treated multiple times to further reduce the impurities content.

In certain embodiments, the pre-leaching composition according to the subject invention is effective due to amphiphiles-mediated penetration of the metal source. In some embodiments, the sophorolipid or other biosurfactant serves as a vehicle for facilitating the transport of a metal. For example, in some embodiments, a sophorolipid will form a micelle containing the metal, wherein the micelle is less than about 100 μm , less than about 10 μm , less than about 1 μm , less than about 100 nm, less than about 50 nm, less than about 25 nm, less than about 15 nm, less than about 10 nm, less than about 5 nm, or less than about 2 nm in size. The small size and amphiphilic properties of the micelle allow for enhanced penetration into the metal source so that greater contact can be made with metal therein.

In one embodiment, the method comprises crushing, grinding or pulverizing the metal source into smaller particles, for example, less than about 1 m, about 75 cm, about 50 cm, about 25 cm, about 10 cm, about 75 mm, about 50 mm, about 25 mm, about 10 mm, about 5 mm, about 1 mm, about 100 μm , about 10 μm , about 1 μm , about 500 nm, about 100 nm, about 50 nm, about 10 nm, about 5 nm, about 1 nm in diameter, prior to treating with the pre-leaching composition. In certain embodiments, the subject compositions and methods can decrease greenhouse gas emissions during the leaching process by, for example, reducing the amount of grinding necessary, thereby reducing the amount of energy consumed during the grinding process. In certain embodiments, the particles in the powder can be at least about 1 cm, 10 cm, about 25 cm, about 50 cm, about 75 cm, or about 1 m in diameter.

In some embodiments, the metal source is obtained from an ore deposit or other source in a raw form. This raw form can comprise additional materials, or gangue. Thus, in certain embodiments, the method can further comprise, after obtaining the metal source concurrent with the

treatment with the subject pre-leaching compositions, and/or after treating the metal source with the pre-leaching composition, subjecting the metal source to one or more beneficiation processes. The one or more beneficiation processes can include, for example, comminution, scrubbing, washing, screening, flotation, and/or hydrocycloning. In certain embodiments, the subject compositions can be used in one or more beneficiation processes, such as, for example, flotation. The subject compositions can be added to compositions used for flotation. Additionally, the subject compositions can replace, or reduce the use of, one or more components of a composition used for flotation, such as, for example, a chemical surfactant.

Advantageously, in certain embodiments, the pre-leaching composition according to the subject invention provides enhanced or increased efficiency at removing impurities from metal with limited negative environmental impacts. Additionally, the methods of the subject invention do not require complicated equipment or high energy consumption, and production of the pre-leaching composition can be performed on site, for example, at a metal recycling facility, an ore mine, or a leaching site. In certain embodiments, the subject pre-leaching composition can result in a decreased use of chemical surfactants or other potentially harmful chemicals during processing of a metal. Furthermore, the reduced-impurity metal materials produced according to the subject invention can be useful for producing more environmentally-friendly, metal products. Additionally, the subject composition can result in decreased greenhouse gas emissions by increasing the concentrations of metals or minerals in leachates or froths, thereby reducing the energy needed to transport or pump froth or leachate.

Bioleaching Methods

The subject invention provides compositions and methods for the removal and/or isolation of metals from tailings, slag or other sources of metals. The leaching process is enhanced by the incorporation of a biosurfactant in the present bioleaching composition. In certain embodiments, the bioleaching methods can be performed after the pre-leaching methods are performed. In alternative embodiments, the bioleaching methods are performed instead of the pre-leaching methods.

Bioleaching methods can be carried out, *in situ*, in a heap leach pad, a column, or any other laboratory or industrial sized reactor. The metals generally exist in ores as insoluble salts, such as sulfides, oxides, silicates, carbonates, or mixed anion salts.

In some embodiments, the presence of a metal solubilizing agent in the bioleaching composition, for example, an aqueous strong acid/oxidizer solution or an aqueous weak acid/oxidizer solution facilitates conversion of insoluble metal salts into soluble metal salts dissolved in water. In an exemplary embodiment, the strong acid can be sulfuric acid, though other acids can

be employed, and the oxidizer can be, for example, a ferric salt, commonly ferric sulfate, or a ferric salt with an anion equivalent of the acid in full dissociated form.

Generally, the metal source is crushed or milled to increase the surface area, although the generation of a preponderance of fine particles can be problematic, as they can agglomerate and cause undesired channeling when a percolation process is employed. Smaller particles generally result in higher metal yields in shorter process times, though the advantageous throughput and yield must be balanced with the increased energy and costs of producing the finer particles.

The process can be, but is not necessarily, carried out at elevated temperatures. Temperatures of about 60 °C to about 150 °C, or more, can be used. Advantageously, the subject invention is useful even at the lower end of this range, for example from 60 °C to 90 °C.

The bioleaching composition can be agitated to accelerate the process. Flow can either be upward or downward, or a counter current can be employed. The reactor can be agitated to promote the reaction where agitation is by bubbling of a reactive or inert gas, stirring, ultrasonic mixing, piezoelectric agitation or any other mode of mixing or agitation. In certain embodiments, the subject compositions and methods can decrease greenhouse gas emissions during the leaching processes by, for example, increasing the efficiency of the leaching process by increasing the penetration of the acid into a source particle, which can reduce the agitation or mixing time and/or energy used for mixing or agitation.

In certain embodiments, the solution comprising the soluble metal can be separated from the non-solution phase by, for example, decantation, filtration, centrifugation, or any combination thereof.

The amount of acid and oxidizer can be controlled to optimize the metal precipitating when, in a subsequent isolation step, a reducing agent, generally another metal, such as iron, is added to promote the precipitation of a metal of fine particle size. Alternatively, the metal sulfate solution is submitted to electrolysis to plate out the metal.

Specific embodiments of the subject invention are directed to the generation and removal of metals from ore, tailings, slag or other sources of one or more target metals where the metal is leached from the source in the form of a water-soluble salt. The metals generally exist in ores as the reduced metal, such as, but not limited to gold. In the presence of a leaching agent, an aqueous alkali metal, alkali earth metal cyanide, or hydrocyanic acid solution, the metal oxidizes to a metal cyanide salt in aqueous solution phase.

In some embodiments, the method comprises crushing, grinding or pulverizing the ore into smaller particles to, for example, increase the surface area. For example, grinding can achieve particles that are less than about 500 µm in size, or any other size, for example, but not limited to about 100 µm, prior to contacting with the leaching solution comprising the biosurfactant. In other

embodiments, the biosurfactant or a solution comprising the biosurfactant is included with the ore during the crushing of the ore. The aqueous solution can be of any pH that enhances the crushing and surface treatment or transformation structurally or chemically to enhance leaching. In some embodiments, the target metal in a reduced state is not oxidized, but the matrix in which it is embedded is degraded to liberate the target metal as a solid non-solution phase. In certain
5 embodiments, the subject compositions and methods can decrease greenhouse gas emissions during the leaching process by, for example, reducing the amount of crushing, grinding or pulverizing necessary during refining, thereby reducing the amount of energy consumed during the crushing, grinding or pulverizing process. In certain embodiments, the particles can be at least about 100 μm ,
10 about 500 μm , about 0.1 cm, 1 cm, 10 cm, about 25 cm, about 50 cm, about 75 cm, or about 1 m in diameter.

The biosurfactant can improve the process by stabilizing the liquid-solid interface and accelerating the chemical exchange at the liquid-solid interface. In some embodiments, the biosurfactant can form complexes with the metal ions to accelerate desorption of the metal ions from
15 the particle surface. The biosurfactant promotes conversion of any passivating metal sulfide layer by its oxidization and displacement from the surface to enhance the penetration of the acidic oxidant into the ore particles. In certain embodiments, the subject compositions and methods can decrease greenhouse gas emissions during the leaching process by, for example, increasing the penetration of the acidic oxidant into the ore particles, thereby reducing the amount of energy consumed during the
20 leaching process as the process requires less of the acid to be produced and transported relative to a particle that has not been treated with a biosurfactant.

The use of the biosurfactant reduces the use of acid and oxidant in the leaching process and accelerates the process by improving the liquid-solid interface. The consequence can be an efficiency increase that permits, when desired, a lowering in temperatures and pressures, as an
25 alternative to the improved throughputs and yields. In certain embodiments, the subject compositions and methods can decrease greenhouse gas emissions during the leaching process by, for example, increasing the throughput and yields, thereby reducing the amount of energy consumed during the leaching process per unit of yield.

The biosurfactants can be included for acceleration of the process and/or enhancement of
30 yield by the stabilization of the liquid-solid interface for oxidation of the sulfides to soluble metal sulfates in any process including the processes known as:

BioNIC, where microorganisms are used with water and air to leach soluble metals, typically from a stirred tank with ores that contain iron sulfides to provide a source of ferric ions;

Intec Nickel Process (INP), where a halide is included as the oxidant in a process carried
35 out in a heated aqueous suspension of sulfide ores, primarily of nickel, copper, or cobalt;

CESL Nickel Process for the leaching of copper and other metals from sulfide ores at slightly elevated temperatures, about 150 °C, and pressures, about 1,400 kPa, in the presences of acid and oxygen;

5 Activox Process, where ultra-fine grinding is employed to activate the mineral surface for oxidative leaching at about 100 to about 110 °C and about 1,000 kPa, has been used to break the sulfide matrix with the solubilization of metals, such as copper, nickel, and cobalt, and the concentration of gold, silver, and platinum in the leach residue for recovery; and

10 FLSmidth Rapid Oxidative Leach Process (ROL) is a mechano-chemical process, where sulfide ores are treated at about 80 °C and atmospheric pressure using acidic ferric sulphate and an oxygen flow with interstage and/or intrastage attrition.

Metals that are isolated in a solution phase and those isolated in the non-solution phase formed in the process can be different, or in some cases, depending on the solubilizing agent, be the same metal. For example, in some cases, Au can be isolated from the solution phase and in other cases can be isolated from the non-solution phase. Common exemplary solution phase liberated
15 metals, include, but are not limited to Cu, Zn, Ni, Co, Ur, Mn, Li, Al, and Sn. Non-solution phase isolatable metals, or non-metals, include, but are not limited to Au, Ag, Pt, and graphite. These can be isolated more rapidly and/or in higher yield by inclusion of the biosurfactants, and their addition extends to traditional and evolving biomining techniques.

In one embodiment, gold mining using a cyanide process can be improved by the use of a
20 biosurfactant according to the subject invention. The efficiency of exposing the gold surface to the cyanide solution enabled by the biosurfactant allows the minimization of the quantity of cyanide required to generate the Au(CN)₂ with little or no Au or cyanide remaining in the leaching system. In other embodiments, the gold remains in reduced form when embodiments employ an acid and an oxidant that removes a matrix from the reduced metal to liberate gold particles in the non-solution
25 phase.

In some embodiments, the acid leaching of heavy metals from contaminated soil can be carried out using hydrochloric acid/nitric acid, (qua regia) or other acids or acid mixtures in the presence of the biosurfactant. The heavy metals that are advantageously removed from the soil with the use of a biosurfactant include, but are not limited to, As, Co, Cr, Ni, Pb, V, Hg and Zn.

30 In embodiments of the invention where the bioleaching is carried out where the solubilizing agent used is not chemical but is a microorganism that generates the required chemicals, the microorganism can be, for example, *Acetobacter metanolicus*, *Acidianus brierleyi*, *Acidophilum cryptum*, *Acinomucor sp.*, *Alternaria sp.*, *Artrobacter Sp.*, *Aspergillus amstelodami*, *Aspergillus clavatus*, *Aspergillus ficuum*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus ochraceus*,
35 *Bacillus sp.*, *Bacillus megaterium*, *Bacillus polymyxa*, *Brettanomyces lambicus*, *Candida sp.*,

Cerostamella sp., *Chlorella vulgaris*, *Chromobacterium violaceum*, *Cladosporium resinae*, *Clostridium* sp., *Coriolus versicolor*, *Corynebacterium* sp., *Crenothrix* sp., *Cunninghamiella* sp., *Fusarium* sp., *Gallionella* sp., *Gleophyllum trabeum*, *Leptospirillum ferrooxidans*, *Leptospirillum thermoferrooxidans*, *Leptotrix* sp., *Metallogenium* sp., *Metallosphaera sedula*, *Mucor* sp.,
5 *Paecilomyces variotii*, *Penicillium brevicompactum*, *Penicillium cyclopium*, *Penicillium funiculosum*, *Penicillium notatum*, *Penicillium ochrochloron*, *Penicillium oxalicum*, *Penicillium simlicissimum*, *Penicillium spinulosum*, *Penicillium variotii*, *Phanerochaete chrysosporium*, *Pichia* sp., *Pseudomonas putida*, *Rhizopus* sp., *Saccharomyces cerevisiae*, *Sulfobacillus thermosulfidooxidans*, *Sulfobacillus thermosulfidooxidans* sub. *Thermotolerans*, *Sulfobacillus*
10 *thermosulfidooxidans* sub. *Asporogenes*, *Sulfolobus acidocaldarius*, *Sulfolobus ambivalens*, *Sulfolobus solfataricus*, *Sulfolobus thermosulfidooxidans*, *Sulfolobus brierlei*, *Sulfolobus yellowstonii*, *Sulfurococcus* sp., *Thermothrix thipara*, *Thiobacillus* sp., *Thiobacillus acidophilus*, *Thiobacillus albertis*, *Thiobacillus capsulatus*, *Thiobacillus concretivorus*, *Thiobacillus caprinus*, *Thiobacillus delicatus*, *Thiobacillus denitrificans*, *Thiobacillus ferrooxidans*, *Thiobacillus*
15 *intermedius*, *Thiobacillus kabobis*, *Thiobacillus neapolitanus*, *Thiobacillus novellus*, *Thiobacillus organoparus*, *Thiobacillus perometabolis*, *Thiobacillus prosperous*, *Thiobacillus rubellus*, *Thiobacillus tepidarius*, *Thiobacillus thiocynoxidans*, *Thiobacillus thiooxidans*, *Thiobacillus thioparys*, *Thiobacillus versutus*, *Trametes versicolor*, *Trichoderma Harzianum*, *Tichoderma viride*, *Yarrowia lipolytica*, or any other bacteria, fungi, or archaea that can generate an
20 acid, ferric salt, or cyanide.

Methods of Extracting Valuable Minerals with Reduced Greenhouse Gas Emissions

In certain embodiments, the subject invention provides a method for extracting target minerals and/or metals from a source comprising rock, ore, tailings, and/or coal combustion waste.

25 In certain embodiments, the method generally comprises obtaining the coal combustion waste from, e.g., a coal powerplant, a landfill or an impoundment, said coal combustion waste comprising one or more target minerals and/or metals, in addition to gangue; reducing the particle size of the coal combustion waste; applying a beneficiation composition comprising one or more microorganisms and/or microbial growth by-products, to the particles in a liquid medium; separating
30 the target minerals and/or metals from the gangue using froth flotation; and collecting the target minerals and/or metals. In certain embodiments, the method generally comprises obtaining the ores, ore slurries, rocks, or tailings from, for example, a mine or quarry, said ores, ore slurries, rocks, or tailings comprising one or more target minerals and/or metals, in addition to gangue; reducing the particle size of the ores, ore slurries, rocks, or tailings; applying a beneficiation composition
35 comprising one or more microorganisms and/or microbial growth by-products, to the particles in a

liquid medium; separating the target minerals and/or metals from the gangue using froth flotation; and collecting the target minerals and/or metals.

In certain embodiments, the coal combustion waste product that serves as the source of the target mineral or metal is fly ash, bottom ash or boiler slag obtained from a coal combustion plant, a landfill, or a wet or dry impoundment. The coal can be anthracite, bituminous, subbituminous and/or lignite coal. In certain specific embodiments, the coal is anthracite coal.

The subject invention further provides methods for separating and/or extracting particles of a target metal or mineral (e.g., a beneficiary) from a particulate source, wherein a beneficiation composition of the subject invention is applied to the particles in a froth flotation medium, or slurry.

In certain embodiments, prior to froth flotation, the methods comprise subjecting the target-containing source (e.g., fly ash, or ore) to roughing or comminution. Roughing or comminution reduces the size and/or size distribution of the ore, rock, tailings, or fly ash particles and, in some embodiments, liberates some of the target mineral or metal from the ore, rock, tailings, or fly ash gangue materials. This can be achieved via, for example, milling, micronizing, pulverizing or otherwise grinding the ore, rock, tailings, or fly ash particles to particles and/or fines having a desired particle size. In some embodiments, the desired particle size is between 10 μm and 5 mm.

In certain embodiments, the method comprises applying the ore, rock, tailings, or fly ash particles and/or fines to water to form a slurry and aerating the slurry in the presence of a beneficiation composition according to the subject invention. In some embodiments, aeration is provided via a sparging system.

In certain embodiments, the composition serves as a collector to facilitate the attachment of air bubbles to particles of the target metal or mineral, which allows for flotation of the particles of the target metal or mineral (e.g., the concentrate) to the surface of the liquid slurry. The method can be performed, for example, in a tank, vat, column, pool or other vessel.

In certain embodiments, the composition serves as a collector to facilitate the attachment of air bubbles to the gangue particles, which allows for flotation of the gangue to the surface of the liquid slurry. The method can be performed, for example, in a tank, vat, column, pool or other vessel.

In certain embodiments, the beneficiation composition serves as a frother to reduce the surface tension of the liquid slurry. In some embodiments, this promotes the even distribution of air bubbles throughout the slurry to enhance adsorption of the concentrate thereto. The reduced surface tension promotes the formation of a stable froth layer at the surface of the slurry, wherein stability prevents the air bubbles from breaking and dropping the adsorbed concentrate particles back into the slurry. In certain embodiments, the surface froth layer comprises the concentrate particles, which can then flow from or be mechanically skimmed from the slurry and collected.

In certain embodiments, the beneficiation composition serves as a frother to reduce the surface tension of the liquid slurry. In some embodiments, this promotes the even distribution of air bubbles throughout the slurry to enhance adsorption of the gangue thereto. The reduced surface tension promotes the formation of a stable froth layer at the surface of the slurry, wherein stability
5 prevents the air bubbles from breaking and dropping the adsorbed gangue particles back into the concentrate. In certain embodiments, the surface froth layer comprises the gangue particles, which can then flow from or be mechanically skimmed from the slurry and removed from the concentrate.

In certain embodiments, the concentrate or gangue particles are detached from the froth bubbles after collection of the froth. The particles can be washed, dried, incinerated, and/or
10 processed by any other means known in the metallurgical arts. Detachment of the particles can be achieved by, for example, applying shear force, such as through the use of an impeller or other mechanical mixer. In certain embodiments, the separated concentrate particles can be dried using, for example, a rotary dryer, a convection dryer, a conveyer, or a fluidized-bed dryer.

In certain embodiments, the composition can be used in methods of direct flotation and
15 reverse flotation. The use of direct floatation of the target mineral or metal or the reverse flotation of the gangue depends on the, for example, chemical makeup, ionic charge, and density of the particles in the slurry. In certain embodiments, the pH of the slurry of the slurry can be modified, including, by, for example, neutralizing the slurry, using the biosurfactant-based beneficiation composition to enhance the effectiveness flotation methods.

In certain embodiments, the pH of the flotation mixture can be about 2 to about 12, about 4
20 to about 10, about 5 to about 9, or about 6 to about 8. Buffers, and pH regulators, such as carbonates and phosphates, may be used to stabilize pH near a preferred value.

The leftover gangue materials remaining in the slurry, or tailings, can be collected and re-
treated according to the subject methods, if desired, or treated using other ash beneficiation systems
25 to produce, e.g., concrete-grade ash.

In certain embodiments, the biosurfactant-based beneficiation composition can be applied with a traditional chemical collector or frother as a modifier, an adjuvant and/or enhancer for the collector or frother. Advantageously, in certain embodiments, the biosurfactant(s) of the subject composition work in synergy with the collectors and/or frothers to improve their performance. This
30 not only allows for potentially reduced volume usage of harsh chemical reagents, but also enhanced separation yields with reduced time and/or energy expenditure, which can in turn improve the environmental impact of the process.

In certain embodiments, the biosurfactant-based beneficiation composition can be applied before, during, and/or after roughing or comminution. In certain embodiments, the biosurfactant-
35 based beneficiation composition can be applied before roughing or comminution instead of a pH

adjusting agent (e.g., a soda), a starch, an amine, or any combination thereof. In certain embodiments, the biosurfactant-based beneficiation composition can be applied before roughing or comminution in combination with a pH adjusting agent (e.g., a soda), a starch, an amine, or any combination thereof.

5 Thus, in certain embodiments, the subject invention provides methods of enhancing the performance of standard collectors or frothers, wherein the reagents are applied to the froth flotation slurry alongside a biosurfactant and/or a biosurfactant-producing microbial culture according to the subject invention.

10 Advantageously, in one embodiment, the subject methods reduce the amount of refining and processing needed to recover pure or nearly pure metals from rock, ore, tailings, and/or coal combustion waste. The subject method can also be used to reduce the amount of chemical surfactants that are used. Additionally, the present invention can be used without releasing large quantities of inorganic and toxic compounds into the environment. Furthermore, the compositions and methods utilize components that are biodegradable and toxicologically safe, and can be used to
15 reduce the amount of toxic waste produced during metal and mineral extraction, and/or reduce the amount of chemical surfactants that are used.

 In certain embodiments, methods are provided for separating finely liberated target metals from waste gangue using froth flotation. In some embodiments, the method comprises obtaining a target metal from ore by, e.g., crushing and grinding the ore to liberate the mineral particles; applying
20 a collector to the liberated target metal particles, wherein the collector modifies the surface properties of the target metal, selectively binding to the surface and imparting hydrophobicity to the metal particles; aerating the liberated particles in a flotation cell containing water to produce air bubbles, wherein the particles attach to the particles and float to the surface in the form of a froth, and wherein the waste gangue remains under the surface; and separating the froth from the water so
25 that the target metal can be further refined. In certain embodiments, the subject compositions and methods can decrease greenhouse gas emissions during the flotation process by, for example, allowing for a larger diameter particle to be floated, relative to a particle that has not been treated with a biosurfactant, and therefore a decreased amount of grinding is necessary, thereby reducing the amount of energy consumed during the grinding process. In certain embodiments, the particles
30 in the powder can be at least about 0.01 cm, about 0.1 cm, about 1 cm, 10 cm, about 25 cm, about 50 cm, about 75 cm, or about 1 m in diameter.

 In preferred embodiments, the collector is a biosurfactant according to the subject invention. Advantageously, in certain embodiments, the biosurfactant modifies the surface properties of the target metal, selectively binding to the surface and imparting hydrophobicity to the metal to
35 facilitate the attachment of air bubbles. Furthermore, the use of biosurfactants as collectors reduces

and/or eliminates the need for conventional collectors, such as, e.g., xanthates (alkyl dithiocarbonates), dithiophosphinates, and thionocarbamates, which are typically petroleum-derived, toxic, hazardous and/or not biodegradable.

5 Reducing the carbon footprint and/or carbon intensity

In certain embodiment, a unit of liquid, such as a kilogram or a cubic foot or any other unit, is monitored to evaluate the attainment of the goal(s). Preferably, the monitoring is quantitative. In one embodiment, carbon credits are earned as well as other current or future environmental benefits market in which individuals or companies are compensated for engaging regenerative and/or environmentally sustainable practices that reduce the use of fossil fuels that generate carbon dioxide, methane, and nitrous oxide but also, in some embodiments, conserve water in which the carbon intensity (such as described by the Argonne GREET model) is reduced, neutral, or even negative.

A “carbon footprint” may be defined herein as a measure of the total amount of carbon dioxide (CO₂) and other GHGs emitted directly or indirectly by a human activity or accumulated over the full life cycle of a product or service. As just one example, a mined ore that requires more grinding before flotation in order to decrease particle size may have a larger carbon footprint than an alternative product that does requires less grinding before flotation because a larger particle size can be used for flotation.

Carbon footprints can be calculated using a Life Cycle Assessment (LCA) method, the Argonne GREET model, or can be restricted to the immediately attributable emissions from energy use of fossil fuels. An LCA, also known as life cycle analysis, ecobalance, and cradle-to-grave analysis) is the investigation and valuation of the environmental impacts of a given product or service caused or necessitated by its existence. The life cycle concept of the carbon footprint means that it is all-encompassing and includes all possible causes that give rise to carbon emissions. In other words, all direct (on-site, internal) and indirect emissions (off-site, external, embodied, upstream, downstream) need to be taken into account.

Normally, a carbon footprint is expressed as a CO₂ equivalent or in some markets as a carbon intensity (CI) score. Carbon dioxide equivalency is a quantity that describes, for a given mixture and amount of GHG, the amount of CO₂ that would have the same global warming potential (GWP), when measured over a specified timescale (generally, 100 years). Carbon dioxide equivalency thus reflects time-integrated radiative forcing. The carbon dioxide equivalency for a gas is obtained by multiplying the mass and the GWP of the gas. The following units are commonly used:

a) By the UN climate change panel IPCC: billion metric tonnes of CO₂ equivalent (GtCO₂ eq);

- b) In industry: million metric tonnes of carbon dioxide equivalents (MMTCDE);
- c) For vehicles: g of carbon dioxide equivalents/km (gCDE/km).

For example, the GWP for methane is 21 and for nitrous oxide 310. This means that emissions of 1 million metric tonnes of methane and nitrous oxide respectively is equivalent to
5 emissions of 21 and 310 million metric tonnes of carbon dioxide.

Various methods exist in the art for calculating or estimating carbon footprints and may be employed in the subject invention.

Advantageously, in preferred embodiments, the subject invention can be useful for reducing the carbon footprint of mining, refining, or the transportation of materials. Specifically, the subject
10 composition and methods can decrease the amount of energy used in the leaching process or during grinding of mined materials before flotation.

A “reduced carbon footprint” means a negative alteration in the amount of carbon dioxide and other GHGs emitted per unit time over the full life cycle of producing a product, through and until a product is ultimately consumed by human consumers or performing a task (e.g., electricity
15 generation). The negative alteration in CO₂ and/or other GHG emissions can be, for example, at least 0.25%, 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

In some embodiments, the term “carbon footprint” is interchangeable herein with the terms “carbon intensity” and “emission intensity.” Emission intensity is the measure of the emission rate
20 of a given GHG relative to the “intensity” of a specific activity or industrial process (e.g., burning of fuel or the processing of gold ore). The emissions intensity can include emission amount relative to, for example, amount of fuel combusted, yield of refined metal or mineral, amount of a commercial product produced, total distance traveled, and/or number of economic units generated.

Emissions intensity is measured across the entire life cycle of a product. For example, the
25 emissions intensity of fuels is calculated by compiling all of the GHG emissions emitted along the supply chain for a fuel, including all the emissions emitted in exploration, mining, collecting, producing, transporting, distributing, dispensing and burning the fuel.

In addition to reducing the carbon footprint and/or carbon intensity of mining, in some embodiments, the subject invention can be used for reducing the number of carbon credits used by
30 an operator involved in, e.g., mining and refining.

Advantageously, the systems of the subject invention can increase the efficiency and reduce the financial and environmental costs of mining practices. In particular, the compositions and methods utilized according to the subject invention can help in preserving valuable natural resources, such as soil and water, while improving production of commodities.

In one embodiment, the methods and compositions according to the subject invention lead to a decrease in emissions of GHG, such as, for example, CO₂, N₂O and/or CH₄, or atmospheric particulates by at least about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 150%, about 200%, or more, compared to similar leaching or refining processes with untreated mined material.

Monitoring of GHG Emissions

In some embodiments, the systems of the subject invention further involve the monitoring of various inputs and outputs of flotation, leaching, and grinding. For example, following application of a composition of a subject invention to a mined material, factors such as water and fossil fuel usage and generation of GHG emissions or other atmospheric particulates can be monitored. Accordingly, the system can be adjusted throughout implementation to account for changes in these factors and make appropriate adjustments to the inputs.

In some embodiments, monitoring comprises performing one or more measurements to assess the effect of the methods of the subject invention on the generation and/or reduction in generation of GHGs or other atmospheric particulates. In certain embodiments, the measurements assess the effect of the methods of the subject invention on the generation and/or reduction in generation of GHGs or other atmospheric particulates.

Measurements and/or monitoring can be conducted at a certain time point after application of the composition to a mined substance or other site at which flotation or leaching occurs. In some embodiments, the measurements are conducted after about 1 week or less, 2 weeks or less, 3 weeks or less, 4 weeks or less, 30 days or less, 60 days or less, 90 days or less, 120 days or less, 180 days or less, 1 year or less and/or 2 years or less. In preferred embodiments, the measurements are conducted at the end of the grinding of a mined material, after leaching of a mined material, and/or after flotation of a mined material.

Furthermore, the measurements and/or monitoring can be repeated over time. In some embodiments, the measurements are repeated daily, weekly, monthly, bi-monthly, semi-monthly, semi-annually, and/or annually.

In certain embodiments, assessing GHG generation can take the form of measuring GHG emissions from a site. Gas chromatography with electron capture detection is commonly used for testing samples in a lab setting. In certain embodiments, GHG emissions can also be conducted at a site, using, for example, flux measurements and/or other developing analytical tools, including, for example, a spectrometric system.

Measuring GHG emissions can also comprise other forms of direct emissions measurement, gas chromatography-mass spectrometry (GC-MS) and/or analysis of fuel input. Direct emissions

measurements can comprise, for example, identifying polluting operational activities (e.g., fuel-burning automobiles, trains, pumps, or grinders) and measuring the emissions of those activities directly through Continuous Emissions Monitoring Systems (CEMS). Fuel input analysis can comprise calculating the quantity of energy resources used (e.g., amount of electricity, fuel, wood, biomass, etc., consumed) determining the content of, for example, carbon, in the fuel source, and applying that carbon content to the quantity of the fuel consumed to determine the amount of emissions.

In some embodiments, the aspects of the system can be centralized such that they are managed, facilitated and/or performed by a single entity. The entity can be a company or a person who manages, facilitates and/or performs all aspects of producing the compositions of the subject invention, formulation and/or customization of the compositions, transportation and application of the compositions, and monitoring of inputs and outputs throughout the course of the treatment. The central entity can also utilize input and output data collected from a single customer and/or multiple customers to predict and formulate future adjustments to the prescribed flotation or leaching programs.

In some embodiments, the central entity can serve as a general contractor, with sub-contractors performing one or more of the production, formulation/customization, transportation and application of the subject compositions, as well as monitoring.

20 Production of Microbe-Based Products

In certain embodiments, the subject invention provides methods for cultivation of microorganisms and production of microbial metabolites and/or other by-products of microbial growth. The subject invention further utilizes cultivation processes that are suitable for cultivation of microorganisms and production of microbial metabolites on a desired scale. These cultivation processes include, but are not limited to, submerged cultivation/fermentation, solid state fermentation (SSF), and modifications, hybrids and/or combinations thereof.

The microorganisms can be, for example, bacteria, yeast and/or fungi. These microorganisms may be natural, or genetically modified microorganisms. For example, the microorganisms may be transformed with specific genes to exhibit specific characteristics. The microorganisms may also be mutants of a desired strain. As used herein, "mutant" means a strain, genetic variant or subtype of a reference microorganism, wherein the mutant has one or more genetic variations (e.g., a point mutation, missense mutation, nonsense mutation, deletion, duplication, frameshift mutation or repeat expansion) as compared to the reference microorganism. Procedures for making mutants are well known in the microbiological art. For example, UV mutagenesis and nitrosoguanidine are used extensively toward this end.

In certain embodiments, the microbes are capable of producing amphiphilic molecules, enzymes, proteins and/or biopolymers. Microbial biosurfactants, in particular, are produced by a variety of microorganisms such as bacteria, fungi, and yeasts, including, for example, *Agrobacterium* spp. (e.g., *A. radiobacter*); *Arthrobacter* spp.; *Aspergillus* spp.; *Aureobasidium* spp. (e.g., *A. pullulans*); *Azotobacter* (e.g., *A. vinelandii*, *A. chroococcum*); *Azospirillum* spp. (e.g., *A. brasiliensis*); *Bacillus* spp. (e.g., *B. subtilis*, *B. amyloliquefaciens*, *B. pumillus*, *B. cereus*, *B. licheniformis*, *B. firmus*, *B. laterosporus*, *B. megaterium*); *Blakeslea*; *Candida* spp. (e.g., *C. albicans*, *C. rugosa*, *C. tropicalis*, *C. lipolytica*, *C. torulopsis*); *Clostridium* (e.g., *C. butyricum*, *C. tyrobutyricum*, *C. acetobutyricum*, and *C. beijerinckii*); *Campylobacter* spp.; *Cornybacterium* spp.; *Cryptococcus* spp.; *Debaryomyces* spp. (e.g., *D. hansenii*); *Entomophthora* spp.; *Flavobacterium* spp.; *Gordonia* spp.; *Hansenula* spp.; *Hanseniaspora* spp. (e.g., *H. uvarum*); *Issatchenkia* spp.; *Kluyveromyces* spp.; *Meyerozyma* spp. (e.g., *M. guilliermondii*); *Mortierella* spp.; *Mycorrhiza* spp.; *Mycobacterium* spp.; *Nocardia* spp.; *Pichia* spp. (e.g., *P. anomala*, *P. guilliermondii*, *P. occidentalis*, *P. kudriavzevii*); *Phycomyces* spp.; *Phythium* spp.; *Pseudomonas* spp. (e.g., *P. aeruginosa*, *P. chlororaphis*, *P. putida*, *P. fluorescens*, *P. fragi*, *P. syringae*); *Pseudozyma* spp. (e.g., *P. aphidis*); *Ralslonia* spp. (e.g., *R. eulopha*); *Rhodococcus* spp. (e.g., *R. erythropolis*); *Rhodospirillum* spp. (e.g., *R. rubrum*); *Rhizobium* spp.; *Rhizopus* spp.; *Saccharomyces* spp. (e.g., *S. cerevisiae*, *S. bouldarii sequela*, *S. torula*); *Sphingomonas* spp. (e.g., *S. paucimobilis*); *Starmerella* spp. (e.g., *S. bombicola*); *Thraustochytrium* spp.; *Torulopsis* spp.; *Ustilago* spp. (e.g., *U. maydis*); *Wickerhamomyces* spp. (e.g., *W. anomalus*); *Williopsis* spp.; and/or *Zygosaccharomyces* spp. (e.g., *Z. bailii*).

In preferred embodiments, microorganism is a *Starmerella* spp. yeast and/or *Candida* spp. yeast, e.g., *Starmerella (Candida) bombicola*, *Candida apicola*, *Candida batistae*, *Candida floricola*, *Candida riodocensis*, *Candida stellate* and/or *Candida kuoi*. In a specific embodiment, the microorganism is *Starmerella bombicola*, e.g., strain ATCC 22214.

As used herein “fermentation” refers to cultivation or growth of cells under controlled conditions. The growth could be aerobic or anaerobic. In preferred embodiments, the microorganisms are grown using SSF and/or modified versions thereof.

In one embodiment, the subject invention provides materials and methods for the production of biomass (e.g., viable cellular material), extracellular metabolites (e.g., small molecules and excreted proteins), residual nutrients and/or intracellular components (e.g., enzymes and other proteins).

The microbe growth vessel used according to the subject invention can be any fermenter or cultivation reactor for industrial use. In one embodiment, the vessel may have functional controls/sensors or may be connected to functional controls/sensors to measure important factors in

the cultivation process, such as pH, oxygen, pressure, temperature, humidity, microbial density and/or metabolite concentration.

In a further embodiment, the vessel may also be able to monitor the growth of microorganisms inside the vessel (e.g., measurement of cell number and growth phases).
5 Alternatively, a daily sample may be taken from the vessel and subjected to enumeration by techniques known in the art, such as dilution plating technique. Dilution plating is a simple technique used to estimate the number of organisms in a sample. The technique can also provide an index by which different environments or treatments can be compared.

In one embodiment, the method includes supplementing the cultivation with a nitrogen
10 source. The nitrogen source can be, for example, potassium nitrate, ammonium nitrate ammonium sulfate, ammonium phosphate, ammonia, urea, and/or ammonium chloride. These nitrogen sources may be used independently or in a combination of two or more.

The method can provide oxygenation to the growing culture. One embodiment utilizes slow motion of air to remove low-oxygen containing air and introduce oxygenated air. In the case of
15 submerged fermentation, the oxygenated air may be ambient air supplemented daily through mechanisms including impellers for mechanical agitation of liquid, and air spargers for supplying bubbles of gas to liquid for dissolution of oxygen into the liquid.

The method can further comprise supplementing the cultivation with a carbon source. The carbon source is typically a carbohydrate, such as glucose, sucrose, lactose, fructose, trehalose,
20 mannose, mannitol, and/or maltose; organic acids such as acetic acid, fumaric acid, citric acid, propionic acid, malic acid, malonic acid, and/or pyruvic acid; alcohols such as ethanol, propanol, butanol, pentanol, hexanol, isobutanol, and/or glycerol; fats and oils such as soybean oil, canola oil, rice bran oil, olive oil, corn oil, sesame oil, and/or linseed oil; etc. These carbon sources may be used independently or in a combination of two or more.

In one embodiment, growth factors and trace nutrients for microorganisms are included in
25 the medium. This is particularly preferred when growing microbes that are incapable of producing all of the vitamins they require. Inorganic nutrients, including trace elements such as iron, zinc, copper, manganese, molybdenum and/or cobalt may also be included in the medium. Furthermore, sources of vitamins, essential amino acids, and microelements can be included, for example, in the
30 form of flours or meals, such as corn flour, or in the form of extracts, such as yeast extract, potato extract, beef extract, soybean extract, banana peel extract, and the like, or in purified forms. Amino acids such as, for example, those useful for biosynthesis of proteins, can also be included.

In one embodiment, inorganic salts may also be included. Usable inorganic salts can be
35 potassium dihydrogen phosphate, dipotassium hydrogen phosphate, disodium hydrogen phosphate, magnesium sulfate, magnesium chloride, iron sulfate, iron chloride, manganese sulfate, manganese

chloride, zinc sulfate, lead chloride, copper sulfate, calcium chloride, sodium chloride, calcium carbonate, and/or sodium carbonate. These inorganic salts may be used independently or in a combination of two or more.

In some embodiments, the method for cultivation may further comprise adding additional
5 acids and/or antimicrobials in the medium before, and/or during the cultivation process. Antimicrobial agents or antibiotics are used for protecting the culture against contamination.

Additionally, antifoaming agents may also be added to prevent the formation and/or accumulation of foam during submerged cultivation.

The pH of the mixture should be suitable for the microorganism of interest. Buffers, and
10 pH regulators, such as carbonates and phosphates, may be used to stabilize pH near a preferred value. When metal ions are present in high concentrations, use of a chelating agent in the medium may be necessary.

The microbes can be grown in planktonic form or as biofilm. In the case of biofilm, the vessel may have within it a substrate upon which the microbes can be grown in a biofilm state. The
15 system may also have, for example, the capacity to apply stimuli (such as shear stress) that encourages and/or improves the biofilm growth characteristics.

In one embodiment, the method for cultivation of microorganisms is carried out at about 5° to about 100° C, preferably, 15 to 60° C, more preferably, 25 to 50° C. In a further embodiment, the cultivation may be carried out continuously at a constant temperature. In another embodiment, the
20 cultivation may be subject to changing temperatures.

In one embodiment, the equipment used in the method and cultivation process is sterile. The cultivation equipment such as the reactor/vessel may be separated from, but connected to, a sterilizing unit, e.g., an autoclave. The cultivation equipment may also have a sterilizing unit that sterilizes *in situ* before starting the inoculation. Air can be sterilized by methods known in the art.
25 For example, the ambient air can pass through at least one filter before being introduced into the vessel. In other embodiments, the medium may be pasteurized or, optionally, no heat at all added, where the use of low water activity and low pH may be exploited to control undesirable bacterial growth.

In one embodiment, the subject invention further provides a method for producing microbial
30 metabolites such as, for example, biosurfactants, enzymes, proteins, ethanol, lactic acid, beta-glucan, peptides, metabolic intermediates, polyunsaturated fatty acid, and lipids, by cultivating a microbe strain of the subject invention under conditions appropriate for growth and metabolite production; and, optionally, purifying the metabolite. The metabolite content produced by the method can be, for example, at least 20%, 30%, 40%, 50%, 60%, 70 %, 80 %, or 90%.

The microbial growth by-product produced by microorganisms of interest may be retained in the microorganisms or secreted into the growth medium. The medium may contain compounds that stabilize the activity of microbial growth by-product.

5 The biomass content of the fermentation medium may be, for example, from 5 g/l to 180 g/l or more, or from 10 g/l to 150 g/l.

The cell concentration may be, for example, at least 1×10^6 to 1×10^{12} , 1×10^7 to 1×10^{11} , 1×10^8 to 1×10^{10} , or 1×10^9 CFU/mL.

10 The method and equipment for cultivation of microorganisms and production of the microbial by-products can be performed in a batch, a quasi-continuous process, or a continuous process.

In one embodiment, all of the microbial cultivation composition is removed upon the completion of the cultivation (e.g., upon, for example, achieving a desired cell density, or density of a specified metabolite). In this batch procedure, an entirely new batch is initiated upon harvesting of the first batch.

15 In another embodiment, only a portion of the fermentation product is removed at any one time. In this embodiment, biomass with viable cells, spores, conidia, hyphae and/or mycelia remains in the vessel as an inoculant for a new cultivation batch. The composition that is removed can be a cell-free medium or contain cells, spores, or other reproductive propagules, and/or a combination of thereof. In this manner, a quasi-continuous system is created.

20 Advantageously, the method does not require complicated equipment or high energy consumption. The microorganisms of interest can be cultivated at small or large scale on site and utilized, even being still-mixed with their media.

In certain embodiments, the subject invention provides a "microbe-based composition," meaning a composition that comprises components that were produced as the result of the growth of microorganisms or other cell cultures. Thus, the microbe-based composition may comprise the microbes themselves and/or by-products of microbial growth. The microbes may be in a vegetative state, in spore form, in mycelial form, in any other form of propagule, or a mixture of these. The microbes may be planktonic or in a biofilm form, or a mixture of both. The by-products of growth may be, for example, metabolites, cell membrane components, expressed proteins, and/or other cellular components. The microbes may be intact or lysed. The microbes may be present in or removed from the composition. The microbes can be present, with broth in which they were grown, in the microbe-based composition. The cells may be present at, for example, a concentration of at least 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} or more CFU per milliliter of the composition.

The subject invention further provides “microbe-based products,” which are products that are to be applied in practice to achieve a desired result. The microbe-based product can be simply a microbe-based composition harvested from the microbe cultivation process. Alternatively, the microbe-based product may comprise further ingredients that have been added. These additional ingredients can include, for example, stabilizers, acids, buffers, carriers, such as water, salt solutions, or any other appropriate carrier, added nutrients to support further microbial growth, non-nutrient growth enhancers, and/or agents that facilitate tracking of the microbes and/or the composition in the environment to which it is applied. The microbe-based product may also comprise mixtures of microbe-based compositions. The microbe-based product may also comprise one or more components of a microbe-based composition that have been processed in some way such as, but not limited to, filtering, centrifugation, lysing, drying, purification and the like.

One microbe-based product of the subject invention is simply the fermentation medium containing the microorganisms and/or the microbial metabolites produced by the microorganisms and/or any residual nutrients. The product of fermentation may be used directly without extraction or purification. If desired, extraction and purification can be easily achieved using standard extraction and/or purification methods or techniques described in the literature.

The microorganisms in the microbe-based products may be in an active or inactive form, or in the form of vegetative cells, reproductive spores, conidia, mycelia, hyphae, or any other form of microbial propagule. The microbe-based products may also contain a combination of any of these forms of a microorganism.

In one embodiment, different strains of microbe are grown separately and then mixed together to produce the microbe-based product. The microbes can, optionally, be blended with the medium in which they are grown and dried prior to mixing.

The microbe-based products may be used without further stabilization, preservation, and storage. Advantageously, direct usage of these microbe-based products preserves a high viability of the microorganisms, reduces the possibility of contamination from foreign agents and undesirable microorganisms, and maintains the activity of the by-products of microbial growth.

Upon harvesting the microbe-based composition from the growth vessels, further components can be added as the harvested product is placed into containers or otherwise transported for use. The additives can be, for example, buffers, carriers, other microbe-based compositions produced at the same or different facility, viscosity modifiers, preservatives, nutrients for microbe growth, surfactants, emulsifying agents, lubricants, solubility controlling agents, tracking agents, solvents, biocides, antibiotics, pH adjusting agents, chelators, stabilizers, ultra-violet light resistant agents, other microbes and other suitable additives that are customarily used for such preparations.

Optionally, the product can be stored prior to use. The storage time is preferably short. Thus, the storage time may be less than 60 days, 45 days, 30 days, 20 days, 15 days, 10 days, 7 days, 5 days, 3 days, 2 days, 1 day, or 12 hours. In a preferred embodiment, if live cells are present in the product, the product is stored at a cool temperature such as, for example, less than 20° C, 15° C, 10°
5 C, or 5° C. On the other hand, a biosurfactant composition can typically be stored at ambient temperatures.

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CLAIMS

We claim:

1. A method for extracting a target metal or mineral from source particles comprising the target metal or mineral and gangue, the method comprising,
 - a) contacting a microbe-based product comprising a biosurfactant to the source particles;
 - b) reducing the size of the source particles by milling, micronizing, pulverizing or grinding the source particles; and
 - c) applying the source particles to a liquid medium to form a slurry, and supplying air bubbles to the slurry,wherein the target metal or mineral separates from the gangue to form a concentrate, wherein the concentrate attaches to the air bubbles and floats to the surface of the slurry, thereby forming a froth layer comprising the concentrate, wherein the gangue remains in the slurry as tailings, and wherein the reduction of the size of the source particles is less than a source particle that has not been treated with the biosurfactant, thereby increasing the efficiency of flotation and reducing the carbon footprint of flotation.
2. The method of claim 1, wherein the source is coal fly ash, a mine, or a quarry.
3. The method of claim 2, wherein the mine is a coal mine, iron ore mine, copper mine, copper-nickel mine, tin mine, nickel mine, gold mine, silver mine, molybdenum mine, aluminum mine, lead-zinc mine, tungsten mine, zinc mine, ruthenium mine, palladium mine, osmium mine, iridium mine, osmiridium mine, or platinum mine.
4. The method of claim 2, wherein the quarry contains chalk, clay, cinder, coal, sand, gravel, coquina, diabase, gabbro, granite, gritstone, gypsum, limestone, marble, ores, phosphate rock, quartz, sandstone, slate, travertine, or any combination thereof.
5. The method of claim 1, wherein the target metal or mineral is iron or an iron-bearing mineral, gold, silver, a platinum group metal, or a rare earth metal.
6. The method of claim 5, wherein the iron-bearing mineral is hematite, goethite, magnetite, martite, limonite, or any combination thereof.

7. The method of claim 1, wherein the liquid medium comprises water, alcohol, aromatic liquid, phenols, azeotropes, and any combination thereof.
8. The method of claim 1, wherein the air bubbles are supplied to the slurry via an air sparging system.
9. The method of claim 1, further comprising applying a frother and/or a collector to the slurry with the microbe-based product.
10. The method of claim 9, wherein the frother is selected from aliphatic alcohols, cyclic alcohols, propylene oxide and polypropylene oxide, propylene glycol, polypropylene glycol and polypropylene glycol ethers, polyglycol ethers, polyglycol glycerol ethers, polyoxyparaffins, natural oils such as pine oil an alcohol blend which is from the waste stream of the production of 2-ethyl hexanol and any combination thereof.
11. The method of claim 9, wherein the collector is selected from as fuel oil, tar oil, animal oil, vegetable oil, fatty acids, fatty acid esters, fatty amines, hydrophobic polymers, neutralized fatty acids, soaps, amine compounds, petroleum-based oily compounds (such as diesel fuels, decant oils, and light cycle oils, kerosene or fuel oils), an organic collector (e.g., xanthates, xanthogen formates, thionocarbamates, dithiophosphates (including sodium, zinc and other salts of dithiophosphates), and mercaptans (including mercaptobenzothiazole), ethyl octylsulfide), and any combination thereof.
12. The method of claim 1, wherein the microbe-based product serves as a frother or a collector.
13. The method of claim 1, further comprising separating the concentrate from the froth layer using mechanical force.
14. The method of claim 13, further comprising drying the concentrate to obtain the target metal or mineral.
15. The method of claim 1, further comprising applying the microbe-based product comprising the biosurfactant to the slurry.
16. The method of claim 1, wherein the biosurfactant is a glycolipid and/or a yeast culture comprising a glycolipid.

17. The method of claim 16, wherein the yeast culture is a *Starmerella* sp. and/or a *Candida* sp. yeast.
18. The method of claim 16, wherein the glycolipid is a sophorolipid, mannosylerythritol lipid, trehalose lipid, rhamnolipid, or any combination thereof.
19. The method of claim 18, wherein the sophorolipid is a linear sophorolipid or a lactonic sophorolipid.
20. The method of claim 1, further comprising measuring the amount of the fossil fuel used to reduce the size of the source particles or transport the source particles or the slurry, wherein the amount of the fossil fuel used to reduce the size of the source particles or transport the source particles or the slurry is reduced when compared to source particles or a slurry that have not been treated with the biosurfactant.
21. A method of bioleaching, comprising:
- a) providing a metal source particle comprising at least one target metal;
 - b) combining the metal source particle with a bioleaching composition comprising:
 - a biosurfactant; and
 - an acid;
 - c) mixing the combined metal source particle and the bioleaching composition;
 - d) reacting the metal source with the bioleaching composition to form a soluble metal comprising solution and a non-solution phase; and
 - e) separating the soluble metal comprising solution from the non-solution phase, wherein the biosurfactant enhances the penetration of the acid into the metal source particle, thereby reducing the greenhouse gas emissions of the mixing of the combined metal source particle and the bioleaching composition, relative to mixing of a metal source that has not been combined with the biosurfactant and an acid.
22. The method according to claim 21, wherein the biosurfactant is a glycolipid and/or a yeast culture comprising a glycolipid.
23. The method according to claim 22, wherein the yeast culture is a *Starmerella* sp. and/or a *Candida* sp. yeast.
24. The method of claim 22, wherein the glycolipid is a sophorolipid, mannosylerythritol lipid, trehalose lipid, rhamnolipid, or any combination thereof.

25. The method of claim 24, wherein the sophorolipid is a linear sophorolipid or a lactonic sophorolipid.
26. The method according to claim 21, wherein the acid is sulfuric acid, hydrochloric acid, nitric acid, phosphoric acid, and/or hydrocyanic acid.
27. The method according to claim 21, wherein the bioleaching composition further comprises an oxidant.
28. The method according to claim 27, wherein the oxidant is ferric chloride, chlorine, bromine, and/or oxygen.
29. The method according to claim 21, wherein the soluble metal comprising solution comprises aluminum, arsenic, cerium, cesium, chromium, cobalt, copper, dysprosium, erbium, europium, gadolinium, gallium, germanium, gold, hafnium, holmium, indium, iridium, lanthanum, lead, lithium, lutetium, magnesium, manganese, mercury, neodymium, nickel, niobium, palladium, platinum, praseodymium, rhodium, rubidium, ruthenium, samarium, scandium, silver, tantalum, tellurium, terbium, thallium, tin, titanium, tungsten, uranium, vanadium, vanadium, ytterbium, yttrium, zinc, zirconium, or any mixture thereof.
30. The method according to claim 21, wherein the non-solution phase comprises gold, silver, platinum, or any mixture thereof.
31. The method according to claim 21, wherein mixing comprises stirring, bubbling of a reactive or inert gas, ultrasonic mixing, piezoelectric agitation, or any combination thereof.
32. The method according to claim 21, wherein separating comprises, decantation, filtration, centrifugation, or any combination thereof.
33. The method according to claim 21, wherein the metal source particle is ore, mine tailings, slag, electronic waste, or any combination thereof.
34. The method according to claim 21, wherein the metal source particle comprises particles of 10 cm or less in diameter.

35. The method according to claim 21, further comprising contacting a pre-leaching composition comprising a biosurfactant with the metal source particle for a period of time before the metal source particle is contacted with the bioleaching composition to yield a mixture comprising a treated metal source and an impurity.

36. The method according to claim 35, wherein the pre-leaching composition is in liquid form, and the contacting comprises stirring, shaking, and/or bubbling the mixture for a time period of about 1 minute to about 48 hours.

37. The method according to claim 36, further comprising separating the impurity and the treated metal source from the mixture to obtain a reduced-impurity metal-containing material.

38. The method according to claim 37, wherein the impurity is present in the liquid phase, the reduced-impurity metal-containing material is in a foam that is formed on the surface of the liquid and the foam containing the reduced-impurity metal-containing material is removed from the liquid.

39. The method of claim 21, wherein the separated soluble metal comprising solution has a higher concentration of the target metal when compared to a metal source particle that has not been treated with the biosurfactant.

40. The method of claim 21, further comprising transporting the separated soluble metal comprising solution.

41. The method of claim 40, further comprising measuring the amount of a fossil fuel used to transport the separated soluble metal comprising solution, wherein the amount of the fossil fuel used is reduced when compared to a separated soluble metal comprising solution that has not been treated with the biosurfactant during a leaching process.

42. The method of claim 21, further comprising measuring the amount of a fossil fuel used to mix the combined metal source particle and the bioleaching composition.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/029264

A. CLASSIFICATION OF SUBJECT MATTER B03D 1/002(2006.01)i; B03D 1/02(2006.01)i; B03D 1/08(2006.01)i 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) B03D 1/002(2006.01); B02C 23/30(2006.01); B03D 1/016(2006.01); C05B 17/00(2006.01); C22B 3/06(2006.01); C22B 3/18(2006.01); C22B 4/00(2006.01) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Korean utility models and applications for utility models Japanese utility models and applications for utility models Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) eKOMPASS(KIPO internal) & Keywords: metal, mineral, microbe-based product, biosurfactant, size, source particles, air bubbles, slurry, gangue, conntrate, froth layer, tailings, bioleaching, acid		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5167375 A (DATTA, RABINDER S.) 01 December 1992 (1992-12-01) column 1, lines 13-18; column 5, lines 40-51; column 7, lines 45-47, 53-59, 61-66; column 8, lines 15-20; column 15, lines 57-61; claim 1; and figure 1	1-20
X	US 2022-0055042 A1 (LOCUS IP COMPANY, LLC) 24 February 2022 (2022-02-24) paragraphs [0045], [0073], [0099], [0136], [0201]; and claims 1, 3, 7, 8, 11, 13, 17, 18	21-42
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A	US 2002-0037245 A1 (GALINA, CHOUGINA) 28 March 2002 (2002-03-28) whole document	1-42
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☐ 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 29 August 2024		Date of mailing of the international search report 30 August 2024
Name and mailing address of the ISA/KR Korean Intellectual Property Office 189 Cheongsa-ro, Seo-gu, Daejeon 35208, Republic of Korea Facsimile No. +82-42-481-8578		Authorized officer HEO, Joo Hyung Telephone No. +82-42-481-5373

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US2024/029264

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				US	2020-340076	A1		29 October 2020	
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				ZA	202003763	B		27 October 2021	
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				EP	1179604	A2		13 February 2002	
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				RU	02172786	C1		27 August 2001	
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