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(54) Title: METHODS FOR CONTROLLING OXYGEN CONCENTRATION DURING AEROBIC BIOSYNTHESIS

(57) Abstract: The present disclosure provides methods for controlling oxygen concentration during aerobic biosynthesis, e.g., fermentation. The method may comprise feeding an oxygen-containing gas into a vessel including a fermentation feedstock and reacting the fermentation feedstock with the oxygen-containing gas to form a broth including a gaseous phase dispersed within the broth. The gaseous phase may comprise any unreacted oxygen from the oxygen-containing gas. The method further includes reducing the concentration of the unreacted oxygen in the dispersed gaseous phase to less than the limiting oxygen concentration ("LOC") for flammability before separating the gaseous phase from the fermentation broth. The concentration of the unreacted oxygen in the gaseous phase is reduced by employing oxygen removal schemes or oxygen dilution schemes.

METHODS FOR CONTROLLING OXYGEN CONCENTRATION DURING AEROBIC BIOSYNTHESIS

PRIORITY CLAIM

[0001] The present application claims the benefit of U.S. Provisional Patent Application No. 62/650,585 filed March 30, 2018, which is incorporated herein by reference for all purposes.

FIELD

[0002] The present disclosure generally relates to methods for controlling oxygen concentration during aerobic biosynthesis. In particular, the present disclosure relates to methods for decreasing oxygen concentration below the limiting oxygen concentration ("LOC") in the gaseous phase of a fermentation broth containing a flammable gas such as hydrogen before the gaseous phase separates from the fermentation broth.

BACKGROUND

[0003] In gas fed fermentation, carbon-rich gases such as carbon dioxide, carbon monoxide and methane, are converted by microorganisms into a wide range of products such as fuel, protein, and chemical compounds, for example, alcohols and organic acids. These products are used by industries in the chemical, petrochemical, pharmaceutical, animal feed, environmental and agricultural sectors. Gas fermentation processes may utilize a variety of feedstocks including domestic, industrial, or agricultural waste, thereby reducing reliance on fossil sources of carbon and reducing emission of greenhouse gases. The fermentation process generally operates at lower reaction temperatures and pressures when compared to high temperature and pressure chemical catalytic reactions.

[0004] Microorganisms used in the fermentation process grow under various engineering and physical conditions inside the fermenter such as agitation, mixing, aeration, pressure, shear, temperature, and pH. Some microorganisms grow under anaerobic conditions while others grow under aerobic conditions. For aerobic reactions, air is generally used as the source of oxygen, but oxygen-enriched air or pure oxygen can also be used. It is generally preferable to operate at the highest possible oxygen concentration to maximize oxygen mass transfer and thereby optimize

productivity. This is because the rate of oxygen mass transfer from the gas phase to the liquid phase is the rate-limiting step for most aerobic microbial biosynthetic reactions.

[0005] During aerobic biosynthesis, any unreacted oxygen from the oxygen source, e.g., air, separates from the fermentation broth into the headspace of the bioreactor, e.g., fermenter. The unreacted oxygen mixes with other unreacted gases, e.g., effluent gases, in the headspace of the bioreactor. In certain situations where the feed gas contains potentially flammable components, the sum of the feed gases, e.g., an oxygen-containing gas, a hydrogen-containing gas, and a carbon-dioxide-containing gas, can have an oxygen concentrations greater than the LOC for the composition containing said flammable components e.g., 6 vol.% oxygen concentration for an air/hydrogen system. Any unreacted oxygen in the gaseous mixture in the fermenter headspace and effluent gases may result in flammable mixtures especially when flammable gases (e.g., hydrogen), flammable volatile organic products, or intermediates, are used or produced in the aerobic biosynthesis process. Even when operating at small-scale, e.g., the laboratory, there is still a risk of explosions from the flammable gas mixture, but the extent this risk is mitigated due to the small-scale of the bioreactor and reduced gas volumes. However, when scaling up the size of bioreactors for pilot or commercial use, the risk of flammability and explosion is a major concern for safe operation of the process. Moreover, when designing a large-scale system to operate above the LOC, necessary equipment design features can be extremely capital expensive especially at higher operating pressures, e.g., explosive-proof electronics, explosive-proof valves, thicker steel, etc. can be required.

[0006] Therefore, the need exists for improved control of the oxygen concentration below the limiting oxygen concentration (LOC) for flammability of flammable gas components before the gaseous phase separates from the fermentation broth into the headspace of the bioreactor while achieving acceptable productivity, capital cost (capital efficiency), and operating cost.

SUMMARY

[0007] In some embodiments, the present disclosure is related to a method for controlling oxygen concentration during aerobic biosynthesis including: feeding an oxygen-containing gas into a bioreactor including a fermentation feedstock; reacting the fermentation feedstock with the oxygen-containing gas to form a broth including a gaseous phase dispersed within the broth, the gaseous phase comprising unreacted oxygen from the oxygen-containing gas; reducing the

concentration of the unreacted oxygen in the gaseous phase to less than the limiting oxygen concentration ("LOC") for flammability; and separating the gaseous phase from the broth. In some aspects, the step of reducing the concentration of the unreacted oxygen occurs prior to the step of separating the gaseous phase from the broth. In some aspects, the step of reducing the concentration of the unreacted oxygen in the gaseous phase comprises adsorbing or reacting the unreacted oxygen with an oxygen reduction catalyst. In some aspects, the step of reducing the concentration of the unreacted oxygen in the gaseous phase comprises absorbing the unreacted oxygen in an oxygen-absorbing liquid that is separated from the fermentation broth with a liquid impervious gas membrane. In some aspects, the step of reducing the concentration of the unreacted oxygen in the gaseous phase comprises diluting the unreacted oxygen with a dilution agent. In some aspects, the dilution agent comprises a gas stream comprising one or more of nitrogen, carbon dioxide, and hydrogen. In some aspects, the dilution agent comprises less than 5 vol.% of oxygen, e.g., less than 3 vol.%. In some aspects, the oxygen-containing gas is air. In some aspects, the oxygen-containing gas comprises an oxygen concentration of greater than 21 vol.%. In some aspects, the gaseous phase separated from the broth comprises less than 6 vol.%, e.g., less than 5.5 vol.%, less than 5 vol.%, less than 4.5 vol.%, less than 4 vol.%, less than 3.5 vol.%, less than 3 vol.%, less than 2 vol.%, or less than 1 vol.%, of oxygen. In some aspects, the feedstock comprises a microorganism including *C. necator* or *C. metallidurans*. In some aspects, the bioreactor is selected from the group consisting of a single fermenter, multiple fermenters in series, a stirred-tank fermenter, a non-stirred tank fermenter, a membrane fermenter, a fixed-bed fermenter, a fluidized-bed fermenter, a single autoclave, multiple autoclaves in series, a plug flow fermenter, a pneumatically agitated fermenter, a gas-lift fermenter with an external loop having forced-circulation, a bubble-column fermenter, a fixed (packed) bed column fermenter, a horizontal single fermenter with multiple compartments, and multistage column fermenters. In some aspects, the gaseous phase is separated from the broth to a headspace of the bioreactor. In some aspects, the method further comprises feeding a flammable gas into the bioreactor. In some aspects, the flammable gas comprises hydrogen.

[0008] In some embodiments, the present disclosure is related to a method for controlling oxygen concentration during aerobic biosynthesis including: feeding a fermentation feedstock into a bioreactor comprising a microorganism; feeding a flammable gas into the bioreactor; feeding an oxygen-containing gas into the bioreactor, the oxygen-containing gas comprising an

oxygen concentration greater than 21 vol.%; reacting the fermentation feedstock with the oxygen-containing gas and the flammable gas to form a broth including a gaseous phase dispersed within the broth, the gaseous phase comprising unreacted oxygen from the oxygen-containing gas and/or the flammable gas; reducing the concentration of the unreacted oxygen in the gaseous phase to less than the limiting oxygen concentration ("LOC"); and separating the gaseous phase from the broth to an upper headspace of the bioreactor. In some aspects, the flammable gas comprises hydrogen. In some aspects, the oxygen-containing gas and the flammable gas are continuously fed to the bioreactor in separate feeds. In some aspects, the step of reducing the concentration of the unreacted oxygen in the gaseous phase comprises adsorbing or reacting the unreacted oxygen with an oxygen reduction catalyst or absorbing the unreacted oxygen with an oxygen-absorbing liquid that is separated from the fermentation broth with a liquid impervious gas membrane. In some aspects, the step of reducing the concentration of the unreacted oxygen in the gaseous phase comprises diluting the unreacted oxygen with a dilution agent comprising less than 5 vol.% of oxygen, e.g., less than 3 vol.%.

BRIEF DESCRIPTION OF THE FIGURES

[0009] The present disclosure will be better understood in view of the appended non-limiting figures, in which:

[0010] FIG. 1 shows a graph of decreased oxygen concentration in the effluent by either an oxygen destruction scheme (Option 1) or an oxygen dilution scheme (Option 2) in accordance with embodiments of the present disclosure.

[0011] FIG. 2 shows an oxygen destruction scheme using a solid oxygen reduction catalyst in accordance with embodiments of the present disclosure.

[0012] FIG. 3 shows an oxygen destruction scheme using a liquid impervious gas membrane in accordance with embodiments of the present disclosure.

[0013] FIG. 4 shows an oxygen gas dilution scheme in which the dispersed gas phase within the fermenter is diluted with nitrogen to reduce the oxygen concentration below the LOC in the dispersed gaseous phase in accordance with embodiments of the present disclosure.

DETAILED DESCRIPTION

[0014] The present disclosure is related to methods for controlling oxygen concentration during aerobic biosynthesis, e.g., fermentation. In some aspects, the oxygen concentration in the gaseous phase of the fermentation broth is reduced below the LOC of the flammable gaseous composition, e.g., 6 vol.% for a hydrogen/oxygen mixture, before it separates from the fermentation broth to the headspace of a bioreactor, e.g., a fermenter. The method for controlling oxygen concentration during aerobic biosynthesis may comprise feeding an oxygen-containing gas into a bioreactor including a microorganism and a flammable fermentation feedstock, and reacting the oxygen of the oxygen-containing gas with the microorganism to form a broth including a gaseous phase with unreacted oxygen dispersed within the broth. The gaseous phase dispersed within the broth can also include a flammable gas, e.g., at least a portion of the flammable fermentation feedstock. The method further includes reducing the concentration of the unreacted oxygen in the dispersed gaseous phase to less than the LOC before separating the gaseous phase from the fermentation broth to the headspace of the bioreactor.

Control Parameters for Oxygen Concentrations

[0015] As described herein, oxygen concentration in the bioreactor is controlled to be within specified ranges. The dissolved oxygen concentration in the fermentation broth is controlled to be at least a minimum value required for the microorganism to function. The minimum value is required because the microorganism is aerobic and requires a certain amount of oxygen. The concentration of gaseous oxygen in the headspace of the bioreactor is controlled to operate safely below the LOC utilizing the oxygen controlling schemes mentioned above. In some aspects, the LOC is approximately 6.0 vol.% oxygen in the gaseous mixtures outside of the fermentation broth. As a safety measure, the gaseous oxygen concentration in the headspace may be measured and controlled to be less than 90% of the LOC, e.g., less than 85%, less than 80%, less than 75%, or less than 70%. In some aspects, the gaseous oxygen concentration in the headspace is controlled to be in range from 60% to 95% of the LOC, e.g., from 65% to 90%, from 70% to 85%, from 70% to 80%, or from 75% to 85%.

[0016] The at least one feed stream comprising oxygen-containing gas may be introduced into the bioreactor by a suitable device in order to create microbubbles and enhance the gas-liquid interface between gas phase and bulk liquid. Additionally, gas-liquid mass transfer depends on

the reactor configuration. There are seven general steps of mass transfer of the gases to the reaction site.

1. Diffusion through the bulk gas within a gas bubble to the gas-liquid interface.
2. Movement across the gas-liquid interface.
3. Diffusion of the solute gas through the relatively unmixed liquid region (film) adjacent to the bubble and into the well-mixed bulk liquid.
4. Transport of the solute gas through the bulk liquid to the stagnant film surrounding the cells.
5. Transport through the second unmixed liquid film associated with the cells.
6. Transport across the cell membrane.
7. Transport through the cell to the reaction site.

Gaseous Oxygen Concentration in Headspace

[0017] The upper limit for gaseous oxygen concentration in the headspace of the bioreactor is limited by safety considerations. Typically, the literature quotes a ratio of 7:1:1 or 8:1:1 for $H_2/CO_2/O_2$ (hydrogen/carbon dioxide/oxygen) for the initial gas mixture for optimum growth/production conditions for gas fermentation for *C.necator* (Ishizaki *et al.* 2001), although this ratio may vary due to adjustments and/or reaction needs. This means that the hydrogen/oxygen ratio is within the flammable range for hydrogen and oxygen gas concentrations. The critical oxygen concentration when mixed with hydrogen with carbon dioxide as a diluent is 5.9 vol.% (Jones and Kenny, 1935). Therefore, the LOC of 5.9 vol.% is here defined to be the minimum oxygen concentration at which a flammable gaseous mixture may form with fermentation process mixtures according to the present disclosure. These fermentation process includes a gaseous phase including, for example, oxygen, nitrogen, hydrogen, carbon dioxide and water vapor mixture, which rises to the headspace of the bioreactor, e.g., fermenter. Temperature and pressure conditions in the bioreactor may also influence the relative concentration of components in the headspace.

[0018] Before the gaseous phase in the fermentation broth rises to the headspace of the bioreactor, the unreacted components, e.g., oxygen, nitrogen, hydrogen, carbon dioxide and/or water vapor, are in a dispersed gaseous phase (e.g., gas bubbles) within the fermentation broth. The concentration of the oxygen in the dispersed gaseous phase is reduced below the LOC before it separates from the fermentation broth into the headspace of the reactor and mixes with

other flammable gases. In particular, the oxygen concentration in the gaseous phase is reduced below the LOC of the headspace gaseous mixture, e.g., 6.0 vol.% oxygen. In order to maintain a safety margin, the bioreactor may be operated within 65% to 85% of the LOC, or even less than 65%. In some aspects, the gaseous oxygen concentration in the headspace is controlled to be from 3.5 to 4.5 vol.% oxygen, e.g., from 3.75 to 4.25 vol.%, from 3.85 to 4.15 vol.%, from 3.95 to 4.05 vol.%, or approximately 4 vol.% oxygen. The bioreactor effluent gas also has the same LOC.

[0019] In some aspects, the steps of mass transfer of the gases to the reaction site include diffusion through the bulk gas within gas bubbles to the gas-liquid interface, movement across the gas-liquid interface, diffusion of the solute gas through the relatively unmixed liquid region (film) adjacent to the bubble and into the well-mixed bulk liquid, transport of the solute gas through the bulk liquid to the stagnant film surrounding the cells, transport through the second unmixed liquid film associated with the cells, transport across the cell membrane, and transport through the cell to the reaction site.

[0020] The gas-liquid mass transfer also depends on the fermenter configuration and the gas mixture should be introduced into the fermenter by a suitable device to create small gas bubbles or microbubbles (having high specific surface area) and thereby increase the gas-liquid interfacial surface area available for gas mass transfer. It is desirable to operate at the highest possible oxygen concentration in order to maximize oxygen mass transfer and thereby maximize productivity in the gas fermentation reaction in the fermenter.

[0021] In some aspects, the concentration of unreacted oxygen in the gaseous phase is reduced by adsorbing or reacting the unreacted oxygen with an oxygen reduction catalyst. The oxygen reduction catalyst can be fed or present in the top of the bioreactor in a sufficient quantity to reduce the oxygen concentration in the gaseous phase below the LOC. In some aspects, the oxygen reduction catalyst is a solid oxygen reduction catalyst. The solid oxygen reduction catalyst can be provided in a portion of the bioreactor that is within or above the fermentation broth, e.g., immediately above or adjacent to the fermentation broth, to capture any unreacted oxygen before the separate gas phase forms in the headspace and of the bioreactor. In this way, the bulk gas only forms in the headspace after the gaseous phase of the fermentation broth comes into contact with the reaction zone of the solid oxygen reduction catalyst.

[0022] In some aspects, the concentration of the unreacted oxygen in the gaseous phase is reduced by adsorbing the unreacted oxygen with a liquid impervious gas membrane. The liquid impervious gas membrane includes an oxygen-absorbing liquid zone that prevents the broth and oxygen-absorbing liquid from co-mixing but allows the gas, e.g., from the collapsing gas bubbles in the broth, to escape from the broth through the membrane into the liquid zone. In other words, the liquid impervious gas membrane allows gas to pass through but is impervious to liquid. The liquid impervious gas membrane can be provided in a portion of the bioreactor that is immediately above or adjacent to the fermentation broth to capture any unreacted oxygen before the separate gas phase, e.g., bulk gas phase, forms in the headspace of the bioreactor. As used herein, the "headspace" is a portion of the bioreactor that does not include the fermentation broth, e.g., the volume above the fermentation broth in a vertical bioreactor. In some aspects, the oxygen dilution scheme and/or the oxygen removal (e.g., destruction) scheme is particularly suitable for use in a vertical reactor with a gradient of oxygen concentration, e.g., loop, uplift, or tubular reactor with a vertical separation area.

[0023] In some aspects, the concentration of the unreacted oxygen in the gaseous phase is reduced by diluting the gaseous phase including the unreacted oxygen with a dilution agent. The dilution agent may comprise a low oxygen gas stream that is fed into the bioreactor in a sufficient quantity to reduce the concentration of unreacted oxygen in the gaseous phase below the LOC of the flammable gas components. In some aspects, the dilution agent can be one or more of nitrogen, carbon dioxide, and hydrogen. In some aspects, the dilution agent comprises oxygen at a concentration below the LOC of the oxygen/flammable gas mixture.

[0024] Conventionally, in order to safely operate an aerobic microbial biosynthesis process with an explosive headspace or gas volume, e.g., gas fermentation, bioreactors are designed with stronger walls to withstand the pressure and temperature from deflagration or explosion. In some cases, the walls of the bioreactor may be reinforced, e.g., constructed with a larger width or made from a specific material, to withstand deflagration or explosion. However, utilizing fermenters with stronger walls may increase capital equipment cost and operating costs. Additional strategies include remaining below the flammability limit, separating flammable gases from oxygen, generating hydrogen *in-situ*, or directly using electrons as an energy source. However, these alternative strategies all have disadvantages in terms of economics or productivity, and may not be compatible with microorganisms for fermentation. For example, operating the

bioreactor below the LOC would reduce oxygen mass transfer from the gas phase to the liquid phase and decrease the overall production rate of the end product.

[0025] The inventors have now discovered that decreasing the oxygen concentration in the fermentation broth prior to the gaseous phase separating from the fermentation broth can greatly reduce the flammability of the headspace gas and effluent gas while maintaining high oxygen mass transfer in the fermentation broth. Utilizing an oxygen removal scheme or an oxygen dilution scheme prior to the gaseous phase separating from the fermentation broth reduces the unreacted oxygen concentration below the flammability limit thereby enabling safe operation of the process. It was found that diluting the gaseous phase with an inert gas, e.g., nitrogen, to an oxygen concentration below the LOC can prevent deflagration or explosions in the headspace of the bioreactor. Advantageously, by diluting the gaseous phase late in the fermentation process, e.g., after the microorganism consumes the maximum amount of oxygen, the process maintains the maximum amount of oxygen in the fermentation broth before it separates to the headspace of the bioreactor. It was also found that removing oxygen from the gaseous phase by adsorption or absorption, e.g., using an oxygen reduction catalyst or oxygen-absorbing liquid in conjunction with a liquid impervious gas membrane, can also prevent deflagration or explosions in the headspace of the bioreactor. Beneficially, this also allows the bioreactor design to include a variety of materials, and is not limited to current reinforced bioreactor designs which accommodate controlled explosions.

[0026] The method advantageously controls the oxygen concentration in the fermentation broth to ensure safe operation of the bioreactor while maintaining oxygen concentration for high conversion of carbon-sources by microorganisms. In a typical gas fermentation process, a fermentation feedstock, e.g., gaseous CO2, including a microorganism is mixed with a flammable gas, e.g., hydrogen, and an oxygen-containing gas to form a fermentation broth. The flammable gas and the oxygen-containing gas are in a dispersed gaseous phase, e.g., gas bubbles, in the broth and any unreacted gases eventually rise to the headspace of the bioreactor, e.g., fermenter, as an effluent gas. The “effluent gas” refers to a gaseous mixture of the gases separated from the fermentation broth during the fermentation process. If the oxygen concentration is relatively high, e.g., above the limiting oxygen concentration (“LOC”) for the flammable components in the effluent gas then it is susceptible to combustion. Advantageously, the process reduces the amount of oxygen in the dispersed gaseous phase before it rises to the

upper portion, e.g., the headspace, of the bioreactor to prevent combustion. The present method enables using higher concentrations of excess oxygen in the feed streams to the bioreactor, e.g., above the LOC for the flammable components, to promote higher reaction rates and then either diluting or removing oxygen from the dispersed gaseous phase of the fermentation broth before it separates to form the bulk gas in the headspace of the bioreactor.

[0027] The present method can greatly improve process efficiencies and enable safe operation of a fermentation process. The method utilizes an oxygen-containing gas having a high oxygen concentration above the LOC, e.g., greater than 6 vol.% oxygen in a hydrogen/air mixture, to promote reaction with the microorganism, and then provides a means to remove or dilute the unreacted oxygen prior to the gaseous phase separating from the broth into the headspace. In some aspects, the oxygen-containing gas may comprise greater than 6 vol.% of oxygen, e.g., greater than 10 vol.%, greater than greater than 20 vol.%, greater than 40 vol.%, greater than 60 vol.%, greater than 80 vol.%, greater than 90 vol.%, greater than 95 vol.%, and greater than 99 vol.%. In some aspects, the oxygen-containing gas comprises pure oxygen.

[0028] In some cases, the fermentation process is an air fed fermentation reaction with an aerobic microorganism in a large-scale non-stirred fermenter. The large-scale non-stirred fermenter can include a fermentation broth with a dispersed gaseous phase within the flammability range. The oxygen concentration of the dispersed gaseous phase within the broth is decreased prior to the gaseous phase separating from the broth into the fermenter headspace. The oxygen concentration in the gaseous phase is decreased to a concentration below the LOC for flammability of the flammable components. For example, for a hydrogen-rich stream containing hydrogen concentrations above the lower flammability limit of hydrogen, the oxygen concentration in the gaseous phase is reduced to less than 6 vol.% of oxygen in the dispersed gaseous phase of the fermentation broth. In some aspects, oxygen concentration in the gaseous phase of the fermentation broth is reduced to less than 6 vol.% of oxygen, e.g., less than 5.9 vol.%, less than 5.5 vol.%, less than 5.0 vol.%, less than 4.0 vol.%, less than 3.0 vol.%, less than 2.0 vol.%, less than 1.0 vol.%, less than 0.5 vol.%, less than 0.1 vol.%, less than .01 vol.%. In some aspects, the oxygen concentration in the gaseous phase is controlled to be less than 90% of the LOC for flammability of the gaseous mixture in the headspace, e.g., less than 85%, less than 80%, less than 75%, or less than 70%.

Microorganism

[0029] A microorganism is provided to the bioreactor described herein in order for the aerobic biosynthesis, e.g., fermentation, to occur. For aerobic reactions, air is generally used as the source of oxygen, but oxygen-enriched air or pure oxygen can also be used. It is generally preferable to operate at the highest possible oxygen concentration in the dispersed gas phase within a fermenter to maximize oxygen mass transfer and thereby optimize productivity. This is because the rate of oxygen mass transfer from the gas phase to the liquid phase is a known rate-limiting step for most aerobic microbial biosynthetic reactions. A consequence of having oxygen concentrations higher than the LOC for the gaseous composition containing the flammable components, e.g., greater than 6 vol.% oxygen, is that any unreacted oxygen in the fermenter headspace and effluent gas stream can result in the formation of unsafe flammable mixtures when flammable gases (e.g., hydrogen), flammable volatile organic products, or intermediates are present.

[0030] The microorganism may be *Cupriavidus necator (C. necator*) or an organism with properties similar thereto. *C. necator* (previously called *Hydrogenomonas eutrophus, Alcaligenes eutropha, Ralstonia eutropha,* and *Wautersia eutropha*) is a Gram-negative, flagellated soil bacterium of the Betaproteobacteria class. This hydrogen-oxidizing bacterium is capable of growing at the interface of anaerobic and aerobic environments and easily adapts between heterotrophic and autotrophic lifestyles. Sources of energy for the bacterium include both organic compounds and hydrogen. Additional properties of *C. necator* include microaerophilicity, copper resistance (Makar and Casida; 1987), bacterial predation (Byrd et al., 1985; Sillman & Casida, 1986; Zeph & Casida, 1986) and polyhydrobutyrate (PHB) synthesis. In addition, the cells have been reported to be capable of both aerobic and nitrate dependent anaerobic growth. A non-limiting example of a *C. necator* organism useful in the present disclosure is a *C. necator* of the H16 strain. In one non-limiting embodiment, a *C. necator* host of the H16 strain with at least a portion of the phaC1AB1 gene locus knocked out (ΔphaCAB), as described in U.S. Patent Application Serial No. 15/717,216, teachings of which are incorporated herein by reference, is used. The organism may be selected from non-pathogenic members of the genera *Ralstonia, Wausteria, Cupriavidus, Alcaligenes, Burkholderia* or *Pandoraea.*

Feed Streams

[0031] As described above, oxygen is needed for fermentation to occur and is introduced to the bioreactor via a feed stream. In order to introduce gaseous feed streams into the bioreactor in a safe manner, at least two different continuous streams of feeds are used. At least one continuous feed stream comprises a flammable gas (e.g., hydrogen) and at least one continuous feed stream comprises gaseous oxygen, e.g., an oxygen-containing gas. The at least one continuous stream comprising a flammable gas would comprise the hydrogen gas (flammable gas), may optionally comprise oxygen at a concentration below the limiting oxygen concentration ("LOC") for flammability of that gas stream, and may optionally comprise all or a portion of the CO_2 gas feed. The at least one continuous stream comprising gaseous oxygen may be an air feed stream. Such a stream would not contain hydrogen gas above the lower flammability limit of hydrogen but may optionally comprise all or a portion of the CO_2 gas feed. Each gas feed stream is introduced into the bioreactor by means such as microbubble generators, venturi nozzles, or porous gas spargers. By separating the hydrogen and oxygen into separate feed streams, a flammable gas mixture cannot form in the feed system and gas mixtures containing both hydrogen and oxygen are present only in the small-volume gas bubbles within the fermentation broth and within the headspace and effluent gas stream.

[0032] In some aspects, the oxygen-containing gas, e.g., air, can be fed directly into the fermentation broth. In some aspects, the oxygen-containing gas can be an oxygen-enriched source, e.g., oxygen-enriched air or pure oxygen. In some aspects, the oxygen-containing gas may comprise greater than 6 vol.% of oxygen, e.g., greater than 10 vol.%, greater than 20 vol.%, greater than 40 vol.%, greater than 60 vol.%, greater than 80 vol.%, or greater than 90 vol.%. In some aspects, the oxygen-containing gas may be pure oxygen.

[0033] In the fermentation process, air is generally used as the source of oxygen, but in some cases pure oxygen or oxygen-enriched air can be used. Any unreacted oxygen (along with the nitrogen present in the air) leaves the reactor or reactors in the gaseous effluent. The unreacted oxygen is commonly referred to as oxygen concentration in the effluent gas or "oxygen leakage." Any vaporized products in the gaseous effluent can be condensed and recovered, and the off-gases leave the system to an abatement system. Products remaining in the broth can be recovered from the liquid effluent from the bioreactor.

Bioreactor

[0034] As described herein, the temperature and pressure parameters of the bioreactor may vary, e.g., at pressures from below atmospheric pressure to above atmospheric pressure, and at temperatures from 20 to 50 °C. The type of bioreactor to be used may be selected based on the desired operating temperature and pressure, as well as on additional factors. Examples of the additional factors include whether mechanical agitation or stirring is desirable, whether the microorganism will be immobilized, and how many oxygen addition points are desired. Examples of bioreactors, such as types of gas fermenters include single fermenters, multiple fermenters in series, stirred-tank fermenters, non-stirred-tank fermenters, membrane fermenters, fixed-bed fermenters, fluidized-bed fermenters, single autoclaves, multiple autoclaves in series, plug flow fermenters, pneumatically agitated fermenters such as gas(air)-lift fermenters, with either internal draft tube loop or external loop, gas-lift fermenters with external loop having forced-circulation, bubble-column fermenters, fixed (packed) bed column fermenters, horizontal single fermenters with multiple compartments, and multistage column fermenters. Additionally, fermenters can be operated in batch, fed-batch, and continuous mode.

Removing Oxygen from Fermentation Broth

[0035] As described herein, the fermentation broth comprises the feed streams in combination with the aerobic microorganism in the bioreactor. In some aspects, the feed streams, e.g., the carbon source feed stream, flammable gas-containing stream, and the oxygen-containing gas feed stream, react with the microorganism in the bioreactor to at least partially form the fermentation broth (which may also include other products, byproducts, and other media fed to the bioreactor). The unreacted oxygen, or the oxygen that is not consumed by the microorganism, exists as both dissolved oxygen and gaseous oxygen in a dispersed gaseous phase within the fermentation broth. The same holds true for the other gases that are soluble. The dispersed gaseous phase, containing the unreacted components, e.g., oxygen, nitrogen, hydrogen, carbon dioxide and/or water vapor, rises to the headspace of the bioreactor.

[0036] The concentration of oxygen in the gaseous phase is reduced to less than less than the limiting oxygen concentration ("LOC") for flammability of the flammable components in the dispersed gas composition. As a safety measure, the gaseous phase that rises to the headspace can be measured and controlled to be less than 90% of the LOC, e.g., less than 85%, less than 80%, less than 75%, or less than 70% of the LOC. In some aspects, the gaseous oxygen

concentration in the headspace is controlled to be in range from 60% to 95% of the LOC, e.g., from 65% to 90%, from 70% to 85%, from 70% to 80%, or from 75% to 85%. In some aspects, the LOC should be less than 6.0 vol.% of oxygen, e.g., less than 5.9 vol.%, less than 5.5 vol.%, less than 5.0 vol.%, less than 4.0 vol.%, less than 3.0 vol.%, less than 2.0 vol.%, less than 1.0 vol.%, less than 0.5 vol.%, less than 0.1 vol.%, less than .01 vol.%, or alternatively, no oxygen. In certain aspects, hydrogen is the only flammable gas in the fermentation reaction system. In terms of ranges, the concentration of oxygen in the gaseous phase is reduced to a range from .01 vol.% to 6.0 vol.%, e.g., 0.1 vol.% to 5.9 vol.%, 0.5 vol.% to 5.5 vol.%, 1.0 vol.% to 5.0 vol.%, 2.0 vol.% to 4.0 vol.%, or 3.0 vol.% to 4.0 vol.%.

[0037] FIG. 1 shows a graph of decreased oxygen concentration in the gaseous phase in accordance with embodiments of the present disclosure. During fermentation, the oxygen concentration in the fermentation broth may be greater than the LOC, e.g., 6 vol.% of oxygen for a hydrogen/oxygen mixture. Since the rate of oxygen mass transfer from the gas phase to the liquid phase is the rate-limiting step in the fermentation process, it is generally preferable to supply the fermentation with the highest possible gas phase oxygen concentration to maximize oxygen mass transfer and thereby optimize production of the end product. However, when the gaseous phase of the fermentation broth includes high concentrations of oxygen, this can result in an effluent gas mixture in the headspace of the bioreactor that is a flammable mixture. In certain aspects, the sum of the feed gases into the fermentation broth is greater than the LOC. In some aspects, the present process reduces the oxygen concentration in the dispersed gaseous phase below the LOC, or to a safety margin below the LOC, before it separates from the broth and forms the effluent gases.

[0038] The oxygen concentration in the gaseous phase can be reduced by either an oxygen removal scheme or an oxygen dilution scheme in accordance with embodiments of the present disclosure. In each of these processes, the oxygen concentration of the dispersed gaseous phase is reduced before it separates from the fermentation broth as effluent gases in headspace of the bioreactor. Specifically, the oxygen concentration of the dispersed gaseous phase is reduced below the LOC, or a safety margin below the LOC, thereby preventing deflagration or explosions in the bioreactor when the separate gaseous phase mixture forms the effluent gases. As shown in FIG. 1, the oxygen concentration is reduced by employing an oxygen removal

scheme (Option 1) or an oxygen dilution scheme (Option 2) prior to the gaseous phase separating from the fermentation broth.

[0039] In some aspects, the method of decreasing the oxygen content may comprise an oxygen removal scheme (Option 1). The oxygen removal scheme may comprise removing oxygen from the gaseous phase by adsorption or absorption. In some aspects, an oxygen reduction catalyst is fed into the fermentation broth before the gaseous phase separates from the broth. In some aspects, the oxygen reduction catalyst is a fixed bed installed in a portion of the bioreactor. In some aspects, the fixed bed including the oxygen reduction catalyst is located within, or immediately above, or adjacent to the fermentation broth such that a separate bulk gas phase does not form until above the oxygen reduction catalyst. In some aspects, the oxygen reduction catalyst is located at an interface between the fermentation broth and the headspace. In some aspects, the oxygen reduction catalyst is a solid oxygen reduction catalyst. The solid oxygen reduction catalyst can capture any unreacted oxygen before it mixes with effluent gas in the headspace of the bioreactor.

[0040] In some aspects, the oxygen removal scheme comprises a guard oxidizer for decreasing oxygen levels where potentially flammable gas or vapor mixtures are present. Guard oxidizers are disclosed, for example, in U.S. Patent Nos. 6,888,034 and 9,221,737, and U.S. Patent Publication No. 2016/0176813, which are incorporated herein by reference. The guard oxidizer can be employed with the bioreactor, e.g., within the bioreactor, to reduce the oxygen concentration below a safety margin of the LOC. In some aspects, the guard oxidizer is within, immediately above, or adjacent to the fermentation broth to reduce the oxygen in the gaseous phase before it forms the bulk gas in the headspace of the bioreactor. In some aspects, the guard oxidizer can decrease oxygen content in the gaseous phase of the fermentation broth, the mixture of effluent gases in the headspace, and/or the final off-gas in an aerobic biosynthesis process. Any unreacted oxygen (along with the nitrogen present in the air) leaves the fermenter or fermenters in the gaseous effluent. In addition to decreasing oxygen concentration, the guard oxidizer also provides stability to the process.

[0041] Unfortunately, at oxygen leakage concentration in excess of the limiting oxygen concentration ("LOC"), unsafe flammable mixtures can form in the headspace and effluent gas stream. Therefore, as a margin of safety, the oxygen leakage for a hydrogen containing mixture is usually kept below 4 vol.%. Higher oxygen leakage also means that the air being fed to the

fermenter(s) is not being fully utilized. In other words, the process requires more air, which leads to increased compression cost. In addition, an increased volume of off-gas causes increased cost for off-gas treatment. U.S. Patent No. 3,957,876 (Rapoport & White) teaches a method to reduce oxygen leakage from a cyclohexane oxidation process through the use of a so-called clean up reaction zone.

[0042] FIG. 2 shows an oxygen removal scheme utilizing an oxygen reduction catalyst in accordance with embodiments of the present disclosure. The oxygen reduction catalyst reduces the oxygen concentration from greater than from 4.0 vol.% in the dispersed gaseous phase to less than 4.0 vol.% before the gaseous phase separates from the broth. Fig. 2 shows that the separated gaseous phase in the headspace of the bioreactor has an oxygen concentration less than 4.0 vol.%. In some aspects, the oxygen reduction catalyst reduces the oxygen concertation to a safety margin that is less than 80% of the LOC. In situations where air is utilized as the oxygen-containing gas, unreacted H_2, unreacted CO_2, nitrogen (from air) and water vapor (saturation concentration) will also be present in the effluent gas. The oxygen reduction catalyst can reduce the concentration of oxygen to less than 4.0 vol.%, which is less than 80% of the LOC for flammability.

[0043] In the illustrated embodiment, the oxygen-containing gas stream, e.g., air, is added to the bioreactor at the highest possible oxygen concentrations in order to maximize oxygen mass transfer and thus maximize productivity. However, unreacted oxygen can be removed from the unreacted gases leaving the fermenter as a gaseous effluent using a solid oxygen reduction catalyst. The solid oxygen reduction catalyst can be located in an upper portion of the fermentation broth to remove excess oxygen before the separate effluent gas phase forms in the headspace. In some aspects, the solid oxygen reduction catalyst is located at an interface between the fermentation broth and the headspace of the bioreactor.

[0044] FIG. 3 shows an oxygen removal scheme utilizing an oxygen-absorbing liquid, that is separated from the fermentation broth with a liquid impervious gas membrane, to reduce the oxygen concertation in the gaseous phase of the broth below the LOC in accordance with embodiments of the present disclosure. The liquid impervious gas membrane reduces the oxygen concentration from greater than from 4.0 vol.% in the dispersed gaseous phase to less than 4.0 vol.%. In some aspects, liquid impervious gas membrane reduces the oxygen concentration in gaseous phase by a relative amount greater than 5%, e.g., greater than 10%, greater than 20%,

greater than 40%, greater than 60%, greater than 80%, or greater than 90%. The liquid impervious gas membrane may be located at an upper portion of the fermentation immediately above (adjacent to) the broth, e.g., a reaction zone for capturing the oxygen in the membrane. In some aspects, the liquid impervious gas membrane is located at an interface between the fermentation broth and the headspace of the bioreactor.

[0045] The liquid impervious gas membrane provides a reaction zone comprising an oxygen-absorbing liquid to remove oxygen from the gaseous phase. The liquid impervious gas membrane prevents the broth and oxygen-absorbing liquid from co-mixing, but allows the gas (from the collapsing gas bubbles in the broth) to escape from the broth into the liquid zone.

[0046] FIG. 4 shows an oxygen dilution scheme in accordance with embodiments of the present disclosure. In some aspects, the method of decreasing the oxygen content may comprise an oxygen dilution scheme. The oxygen dilution scheme dilutes the dispersed gas phase within the broth with nitrogen to reduce the oxygen concentration below the LOC of the dispersed gaseous phase. As shown in Fig. 4, nitrogen is fed to the bioreactor to dilute the oxygen in the dispersed gaseous phase before a separate gas phase mixture forms in the headspace and effluent gas. In some aspects, the nitrogen feed stream can be supplied to the bioreactor at an upper portion of the fermentation broth. The oxygen dilution scheme dilutes the dispersed gaseous phase in the fermentation broth prior to separation of the dispersed gas into the headspace and effluent gas with a suitable dilution gas stream, e.g., a gas stream depleted of oxygen, an inert gas stream, or a gas stream that has a high concentration of a flammable gas (e.g., pure hydrogen gas).

[0047] The oxygen dilution scheme comprises diluting the dispersed gaseous phase including the unreacted oxygen with a suitable dilution agent. In some aspects, the dilution agent can be a stream depleted of oxygen or an inert gas stream. In some aspects, the dilution agent may comprise nitrogen, hydrogen, carbon dioxide, or combinations thereof. In some aspects, the dilution agent can be a stream comprising less than 6.0 vol.% of oxygen, e.g., less than 5.9 vol.%, less than 5.5 vol.%, less than 5.0 vol.%, less than 4.0 vol.%, less than 3.0 vol.%, less than 2.0 vol.%, less than 1.0 vol.%, less than 0.5 vol.%, less than 0.1 vol.%, less than .01 vol.%, or alternatively, no oxygen. For example, the dilution agent may consist of an inert gas.

[0048] By decreasing the oxygen concentration to below the LOC for flammability, e.g., 6 vol.% for a hydrogen/air mixture, the method reduces the degree of flammability of the gas mixture and diminishes the risk of deflagration or explosions. In some aspects, the method of decreasing the

oxygen content comprises diluting the dispersed gas phase with an inert gas, e.g., nitrogen, before a separate bulk gas phase forms in the headspace of the bioreactor. The method reduces the oxygen concentration within a safety margin below the LOC for flammability of the gas mixture.

[0049] In some aspects, the dilution agent is introduced to the bioreactor as a recycle stream to the bioreactor, e.g., a recycle stream comprising nitrogen or other gases from the fermentation process. In some aspects, the oxygen concentration is diluted by adding a hydrogen, nitrogen, carbon dioxide recycle stream at an upper portion of the fermentation broth. The unreacted hydrogen and carbon dioxide is recycled to an upper portion of the fermentation broth rather than at the bottom of the fermenter to achieve recycle as well as dilute the oxygen in the gaseous phase of the broth. The dilution agent is fed to the reactor to reduce the oxygen concentration below the LOC for flammability. Without the aforementioned oxygen destruction (e.g., removal) and dilution schemes, bioreactors would need to be built with thicker/stronger walls in order to safely contain the potentially flammable mixture, and such bioreactors would be more expensive.

[0050] In some aspects, the oxygen dilution scheme and the oxygen removal scheme can be used in combination to reduce the oxygen concentration.

Embodiments

[0051] Embodiment 1: A method for controlling oxygen concentration during aerobic biosynthesis, the method comprising: feeding an oxygen-containing gas into a bioreactor containing a microorganism, wherein a flammable gas fermentation feedstock component is within the bioreactor; reacting at least a portion of the oxygen from the oxygen-containing gas with the microorganism; forming a broth including a gaseous phase dispersed within the broth, the gaseous phase comprising unreacted oxygen from the oxygen-containing gas; reducing the concentration of the unreacted oxygen in the gaseous phase to less than the limiting oxygen concentration (LOC) for flammability of the flammable gas feedstock component, wherein the reducing comprises diluting the unreacted oxygen with a dilution agent; and separating the gaseous phase from the broth.

[0052] Embodiment 2: An embodiment of embodiment 1, wherein the dilution agent comprises a gas stream comprising one or more of nitrogen, carbon dioxide, and hydrogen.

[0053] Embodiment 3: An embodiment of embodiment 1 or 2, wherein the dilution agent comprises less than 5 vol.% oxygen.

[0054] Embodiment 4: An embodiment of any embodiment of embodiment 1-3, wherein the oxygen-containing gas comprises greater than 21 vol.% oxygen.

[0055] Embodiment 5: An embodiment of any embodiment of embodiment 1-4, wherein the oxygen-containing gas comprises air.

[0056] Embodiment 6: An embodiment of any embodiment of embodiment 1-5, wherein the gaseous phase separated from the broth comprises oxygen at a concentration less than 85% of the LOC.

[0057] Embodiment 7: An embodiment of any embodiment of embodiment 1-6, wherein the gaseous phase separated from the broth comprises less than 6 vol.% oxygen.

[0058] Embodiment 8: An embodiment of any embodiment of embodiment 1-7, wherein the step of reducing the concentration of the unreacted oxygen occurs prior to the step of separating the gaseous phase from the broth.

[0059] Embodiment 9: An embodiment of any embodiment of embodiment 1-8, wherein the microorganism comprises *C. necator* or *C. metallidurans*.

[0060] Embodiment 10: An embodiment of any embodiment of embodiment 1-9, wherein the bioreactor is selected from the group consisting of a single fermenter, multiple fermenters in series, a stirred-tank fermenter, a non-stirred tank fermenter, a membrane fermenter, a fixed-bed fermenter, a fluidized-bed fermenter, a single autoclave, multiple autoclaves in series, a plug flow fermenter, a pneumatically agitated fermenter, a gas-lift fermenter with an external loop having forced-circulation, a bubble-column fermenter, a fixed (packed) bed column fermenter, a horizontal single fermenter with multiple compartments, and multistage column fermenters.

[0061] Embodiment 11: An embodiment of any embodiment of embodiment 1-10, wherein the separating comprises separating the gaseous phase from the broth to a headspace of the bioreactor.

[0062] Embodiment 12: An embodiment of any embodiment of embodiment 1-11, further comprising feeding a flammable gas composition into the bioreactor.

[0063] Embodiment 13: An embodiment of embodiment 12, wherein the flammable gas composition comprises hydrogen.

[0064] Embodiment 14: An embodiment of any embodiment of embodiment 12 or 13, wherein the oxygen-containing gas and the flammable gas composition are continuously fed to the bioreactor in separate feeds.

[0065] Embodiment 15: A method for controlling oxygen concentration during aerobic biosynthesis, the method comprising: feeding an oxygen-containing gas into a bioreactor containing a microorganism, wherein a flammable gas fermentation feedstock component is within the bioreactor; reacting at least a portion of the oxygen from the oxygen-containing gas with the microorganism; forming a broth including a gaseous phase dispersed within the broth, the gaseous phase comprising unreacted oxygen from the oxygen-containing gas; reducing the concentration of the unreacted oxygen in the gaseous phase to less than the LOC for flammability of the flammable gas feedstock component, wherein the reducing comprises adsorbing or reacting the unreacted oxygen with a solid oxygen catalyst; and separating the gaseous phase from the broth.

[0066] Embodiment 16: An embodiment of embodiment 15, wherein oxygen-containing gas comprises greater than 21 vol.% oxygen.

[0067] Embodiment 17: An embodiment of embodiment 15 or 16, wherein the oxygen-containing gas comprises air.

[0068] Embodiment 18: An embodiment of any embodiment of embodiment 15-17, wherein the gaseous phase separated from the broth comprises oxygen at a concentration less than 85% of the LOC.

[0069] Embodiment 19: An embodiment of any embodiment of embodiment 15-18, wherein the gaseous phase separated from the broth comprises less than 6 vol.% oxygen.

[0070] Embodiment 20: An embodiment of any embodiment of embodiment 15-19, wherein the step of reducing the concentration of the unreacted oxygen occurs prior to the step of separating the gaseous phase from the broth.

[0071] Embodiment 21: An embodiment of any embodiment of embodiment 15-20, wherein the microorganism comprises *C. necator* or *C. metallidurans*.

[0072] Embodiment 22: An embodiment of any embodiment of embodiment 15-21, wherein the bioreactor is selected from the group consisting of a single fermenter, multiple fermenters in series, a stirred-tank fermenter, a non-stirred tank fermenter, a membrane fermenter, a fixed-bed fermenter, a fluidized-bed fermenter, a single autoclave, multiple autoclaves in series, a plug flow fermenter, a pneumatically agitated fermenter, a gas-lift fermenter with an external loop having forced-circulation, a bubble-column fermenter, a fixed (packed) bed column fermenter, a horizontal single fermenter with multiple compartments, and multistage column fermenters.

[0073] Embodiment 23: An embodiment of any embodiment of embodiment 15-22, wherein separating comprises separating the gaseous phase from the broth to a headspace of the bioreactor.

[0074] Embodiment 24: An embodiment of any embodiment of embodiment 15-23, further comprising feeding a flammable gas composition into the bioreactor.

[0075] Embodiment 25: An embodiment of embodiment 24, wherein the flammable gas composition comprises hydrogen.

[0076] Embodiment 26: An embodiment of embodiment 24 or 25, wherein the oxygen-containing gas and the flammable gas composition are continuously fed to the bioreactor in separate feeds.

[0077] Embodiment 27: A method for controlling oxygen concentration during aerobic biosynthesis, the method comprising: feeding an oxygen-containing gas into a bioreactor containing a microorganism, wherein a flammable gas fermentation feedstock component is within the bioreactor; reacting at least a portion of the oxygen from the oxygen-containing gas with the microorganism; forming a broth including a gaseous phase dispersed within the broth, the gaseous phase comprising unreacted oxygen from the oxygen-containing gas; reducing the concentration of the unreacted oxygen in the gaseous phase to less than the LOC for flammability of the flammable gas feedstock component, wherein the reducing comprises absorbing the unreacted oxygen in an oxygen absorbing liquid; and separating the gaseous phase from the broth.

[0078] Embodiment 28: An embodiment of embodiment 27, wherein oxygen-containing gas comprises greater than 21 vol.% oxygen.

[0079] Embodiment 29: An embodiment of embodiment 27 or 28, wherein the oxygen-containing gas comprises air.

[0080] Embodiment 30: An embodiment of any embodiment of embodiment 27-29, wherein the gaseous phase separated from the broth comprises oxygen at a concentration less than 85% of the LOC.

[0081] Embodiment 31: An embodiment of any embodiment of embodiment 27-30, wherein the gaseous phase separated from the broth comprises less than 6 vol.% oxygen.

[0082] Embodiment 32: An embodiment of any embodiment of embodiment 27-31, wherein the step of reducing the concentration of the unreacted oxygen occurs prior to the step of separating the gaseous phase from the broth.

[0083] Embodiment 33: An embodiment of any embodiment of embodiment 27-32, wherein the microorganism comprises *C. necator* or *C. metallidurans*.

[0084] Embodiment 34: An embodiment of any embodiment of embodiment 27-33, wherein the bioreactor is selected from the group consisting of a single fermenter, multiple fermenters in series, a stirred-tank fermenter, a non-stirred tank fermenter, a membrane fermenter, a fixed-bed fermenter, a fluidized-bed fermenter, a single autoclave, multiple autoclaves in series, a plug flow fermenter, a pneumatically agitated fermenter, a gas-lift fermenter with an external loop having forced-circulation, a bubble-column fermenter, a fixed (packed) bed column fermenter, a horizontal single fermenter with multiple compartments, and multistage column fermenters.

[0085] Embodiment 35: An embodiment of any embodiment of embodiment 27-34 wherein the separating comprises separating the gaseous phase from the broth to a headspace of the bioreactor.

[0086] Embodiment 36: An embodiment of any embodiment of embodiment 27-35, further comprising feeding a flammable gas composition into the bioreactor.

[0087] Embodiment 37: An embodiment of embodiment 36, wherein the flammable gas composition comprises hydrogen.

[0088] Embodiment 38: An embodiment of embodiment 36 or 37, wherein the oxygen-containing gas and the flammable gas composition are continuously fed to the bioreactor in separate feeds.

[0089] While the disclosure has been described in detail, modifications within the spirit and scope of the disclosure will be readily apparent to those of skill in the art. It should be understood that aspects of the disclosure and portions of various embodiments and various features recited above and/or in the appended claims may be combined or interchanged either in whole or in part. In the foregoing descriptions of the various embodiments, those embodiments which refer to another embodiment may be appropriately combined with other embodiments as will be appreciated by one of ordinary skill in the art. Furthermore, those of ordinary skill in the art will appreciate that the foregoing description is by way of example only, and is not intended

to limit the disclosure. All U.S. patents and publications cited herein are incorporated by reference in their entirety. References recited herein are provided with full details as follows:

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What is claimed is:

1. A method for controlling oxygen concentration during aerobic biosynthesis, the method comprising:

feeding an oxygen-containing gas into a bioreactor containing a microorganism, wherein a flammable gas fermentation feedstock component is within the bioreactor;

reacting at least a portion of the oxygen from the oxygen-containing gas with the microorganism;

forming a broth including a gaseous phase dispersed within the broth, the gaseous phase comprising unreacted oxygen from the oxygen-containing gas;

reducing the concentration of the unreacted oxygen in the gaseous phase to less than the limiting oxygen concentration (LOC) for flammability of the flammable gas feedstock component, wherein the reducing comprises diluting the unreacted oxygen with a dilution agent; and

separating the gaseous phase from the broth.

2. The method of claim 1, wherein the dilution agent comprises a gas stream comprising one or more of nitrogen, carbon dioxide, and hydrogen.

3. The method of claim 1 or 2, wherein the dilution agent comprises less than 5 vol.% oxygen.

4. The method of any one of claims 1-3, wherein the oxygen-containing gas comprises greater than 21 vol.% oxygen.

5. The method of any one of claims 1-4, wherein the oxygen-containing gas comprises air.

6. The method of any one of claim 1-5, wherein the gaseous phase separated from the broth comprises oxygen at a concentration less than 85% of the LOC.

7. The method of any one of claims 1-6, wherein the gaseous phase separated from the broth comprises less than 6 vol.% oxygen.

8. The method of any one of claims 1-7, wherein the step of reducing the concentration of the unreacted oxygen occurs prior to the step of separating the gaseous phase from the broth.

9. The method of any one of claims 1-8, wherein the microorganism comprises *C. necator* or *C. metallidurans*.

10. The method of any one of claims 1-9, wherein the bioreactor is selected from the group consisting of a single fermenter, multiple fermenters in series, a stirred-tank fermenter, a non-stirred tank fermenter, a membrane fermenter, a fixed-bed fermenter, a fluidized-bed fermenter, a single autoclave, multiple autoclaves in series, a plug flow fermenter, a pneumatically agitated fermenter, a gas-lift fermenter with an external loop having forced-circulation, a bubble-column fermenter, a fixed (packed) bed column fermenter, a horizontal single fermenter with multiple compartments, and multistage column fermenters.

11. The method of any one of claims 1-10, wherein the separating comprises separating the gaseous phase from the broth to a headspace of the bioreactor.

12. The method of any one of claims 1-11, further comprising feeding a flammable gas composition into the bioreactor.

13. The method of claim 12, wherein the flammable gas composition comprises hydrogen.

14. The method of claim 12 or 13, wherein the oxygen-containing gas and the flammable gas composition are continuously fed to the bioreactor in separate feeds.

15. A method for controlling oxygen concentration during aerobic biosynthesis, the method comprising:

feeding an oxygen-containing gas into a bioreactor containing a microorganism, wherein a flammable gas fermentation feedstock component is within the bioreactor;

reacting at least a portion of the oxygen from the oxygen-containing gas with the microorganism;

forming a broth including a gaseous phase dispersed within the broth, the gaseous phase comprising unreacted oxygen from the oxygen-containing gas;

reducing the concentration of the unreacted oxygen in the gaseous phase to less than the limiting oxygen concentration (LOC) for flammability of the flammable gas feedstock component, wherein the reducing comprises adsorbing or reacting the unreacted oxygen with a solid oxygen catalyst; and

separating the gaseous phase from the broth.

16. The method of claim 15, wherein oxygen-containing gas comprises greater than 21 vol.% oxygen.

17. The method of claim 15 or 16, wherein the oxygen-containing gas comprises air.

18. The method of any one of claim 15-17, wherein the gaseous phase separated from the broth comprises oxygen at a concentration less than 85% of the LOC.

19. The method of any one of claims 15-18, wherein the gaseous phase separated from the broth comprises less than 6 vol.% oxygen.

20. The method of any one of claims 15-19, wherein the step of reducing the concentration of the unreacted oxygen occurs prior to the step of separating the gaseous phase from the broth.

21. The method of any one of claims 15-20, wherein the microorganism comprises *C. necator* or *C. metallidurans*.

22. The method of any one of claims 15-21, wherein the bioreactor is selected from the group consisting of a single fermenter, multiple fermenters in series, a stirred-tank fermenter, a non-stirred tank fermenter, a membrane fermenter, a fixed-bed fermenter, a fluidized-bed fermenter, a single autoclave, multiple autoclaves in series, a plug flow fermenter, a pneumatically agitated

fermenter, a gas-lift fermenter with an external loop having forced-circulation, a bubble-column fermenter, a fixed (packed) bed column fermenter, a horizontal single fermenter with multiple compartments, and multistage column fermenters.

23. The method of any one of claims 15-22, wherein separating comprises separating the gaseous phase from the broth to a headspace of the bioreactor.

24. The method of any one of claims 15-23, further comprising feeding a flammable gas composition into the bioreactor.

25. The method of claim 24, wherein the flammable gas composition comprises hydrogen.

26. The method of claim 24 or 25, wherein the oxygen-containing gas and the flammable gas composition are continuously fed to the bioreactor in separate feeds.

27. A method for controlling oxygen concentration during aerobic biosynthesis, the method comprising:

feeding an oxygen-containing gas into a bioreactor containing a microorganism, wherein a flammable gas fermentation feedstock component is within the bioreactor;

reacting at least a portion of the oxygen from the oxygen-containing gas with the microorganism;

forming a broth including a gaseous phase dispersed within the broth, the gaseous phase comprising unreacted oxygen from the oxygen-containing gas;

reducing the concentration of the unreacted oxygen in the gaseous phase to less than the LOC for flammability of the flammable gas feedstock component, wherein the reducing comprises absorbing the unreacted oxygen in an oxygen absorbing liquid; and

separating the gaseous phase from the broth.

28. The method of claim 27, wherein oxygen-containing gas comprises greater than 21 vol.% oxygen.

29. The method of claim 27 or 28, wherein the oxygen-containing gas comprises air.

30. The method of any one of claims 27-29, wherein the gaseous phase separated from the broth comprises oxygen at a concentration less than 85% of the LOC.

31. The method of any one of claims 27-30, wherein the gaseous phase separated from the broth comprises less than 6 vol.% oxygen.

32. The method of any one of claims 27-31, wherein the step of reducing the concentration of the unreacted oxygen occurs prior to the step of separating the gaseous phase from the broth.

33. The method of any one of claims 27-32, wherein the microorganism comprises *C. necator* or *C. metallidurans*.

34. The method of any one of claims 27-33, wherein the bioreactor is selected from the group consisting of a single fermenter, multiple fermenters in series, a stirred-tank fermenter, a non-stirred tank fermenter, a membrane fermenter, a fixed-bed fermenter, a fluidized-bed fermenter, a single autoclave, multiple autoclaves in series, a plug flow fermenter, a pneumatically agitated fermenter, a gas-lift fermenter with an external loop having forced-circulation, a bubble-column fermenter, a fixed (packed) bed column fermenter, a horizontal single fermenter with multiple compartments, and multistage column fermenters.

35. The method of any one of claims 27-34 wherein the separating comprises separating the gaseous phase from the broth to a headspace of the bioreactor.

36. The method of any one of claims 27-35, further comprising feeding a flammable gas composition into the bioreactor.

37. The method of claim 36, wherein the flammable gas composition comprises hydrogen.

38. The method of claim 36 or 37, wherein the oxygen-containing gas and the flammable gas composition are continuously fed to the bioreactor in separate feeds.

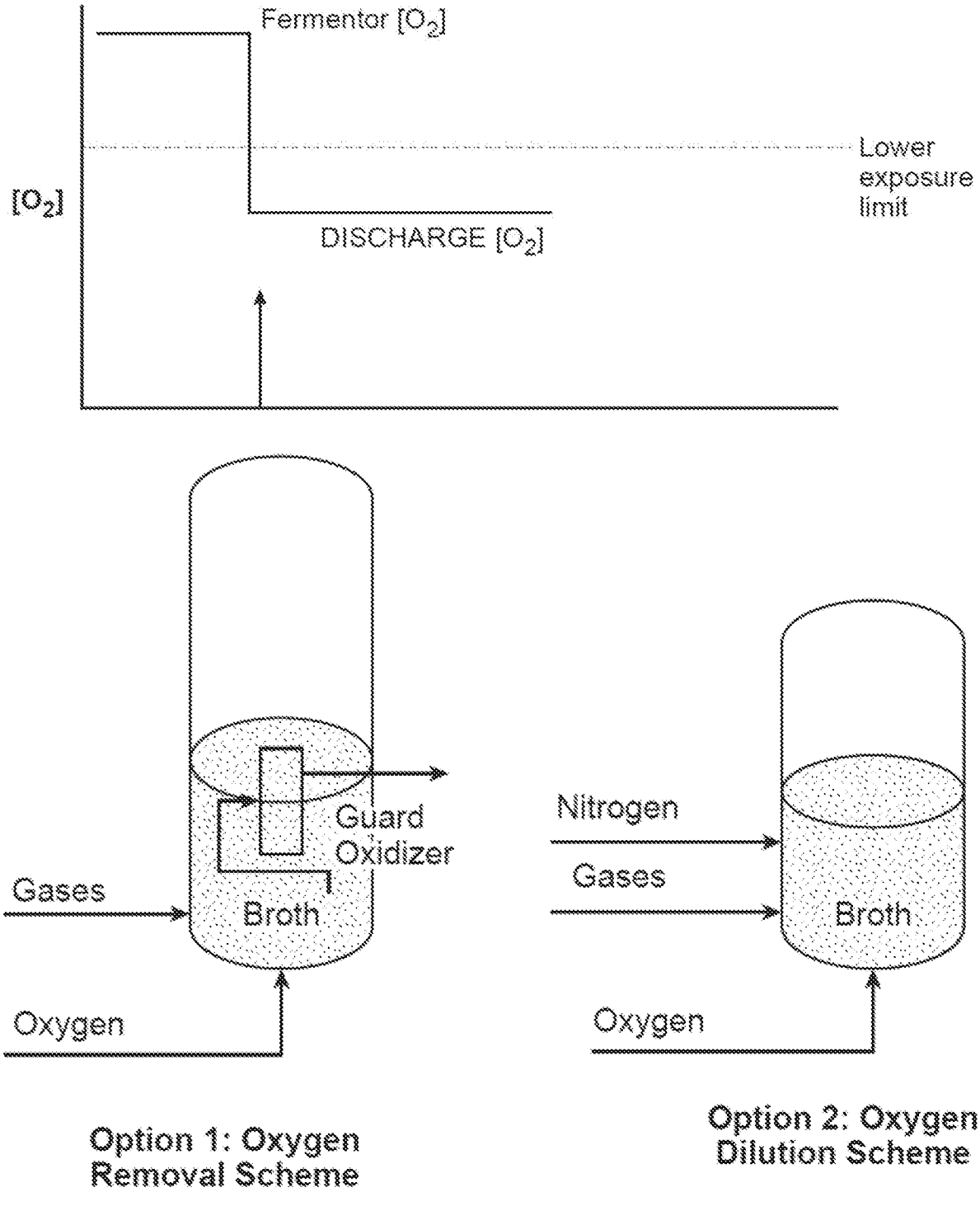
Fermentor [O2]
[O2]
Lower exposure limit
DISCHARGE [O2]
Guard Oxidizer
Gases
Broth
Oxygen
Option 1: Oxygen Removal Scheme
Nitrogen
Gases
Broth
Oxygen
Option 2: Oxygen Dilution Scheme

FIG. 1

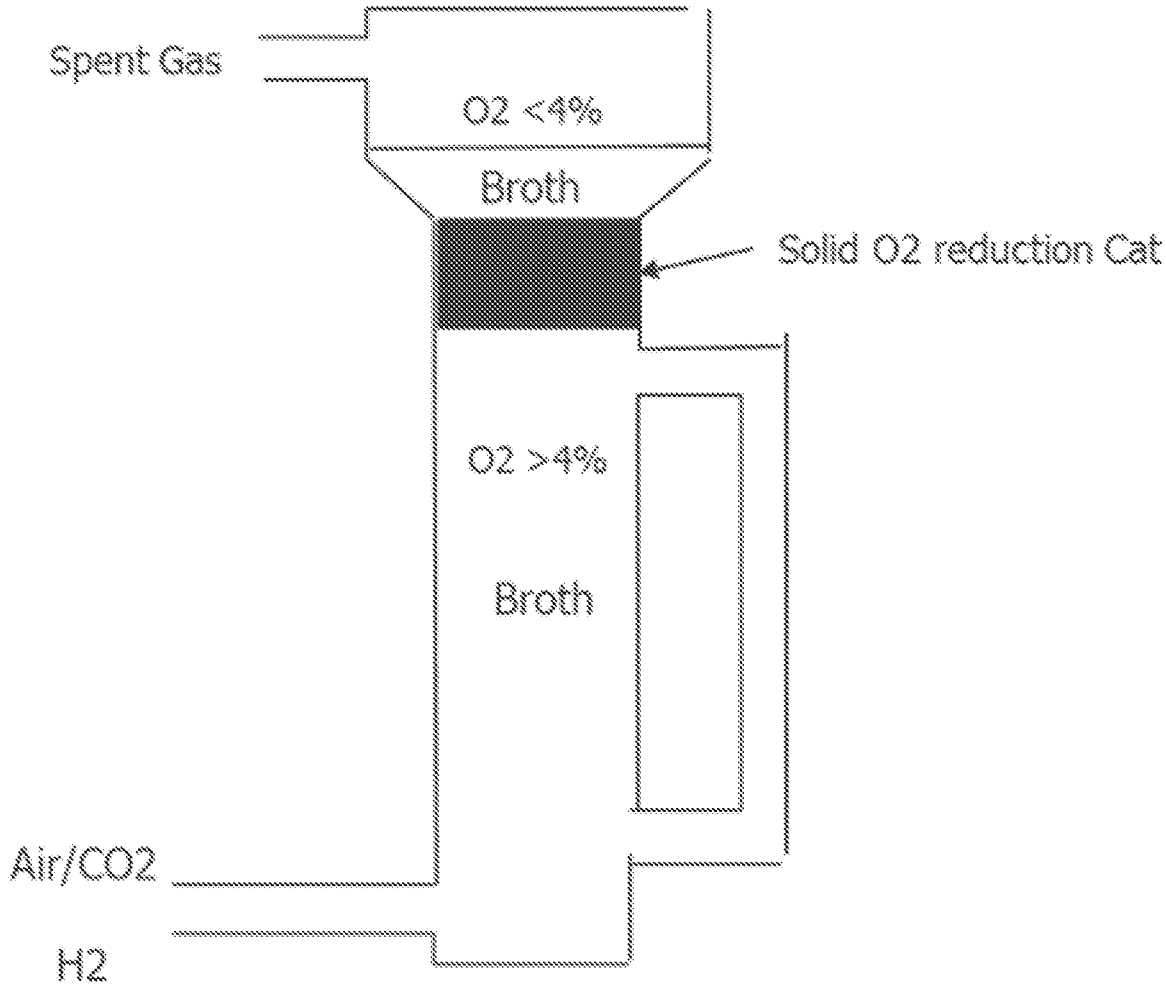
Spent Gas
O2 <4%
Broth
Solid O2 reduction Cat
O2 >4%
Broth
Air/CO2
H2

FIG. 2

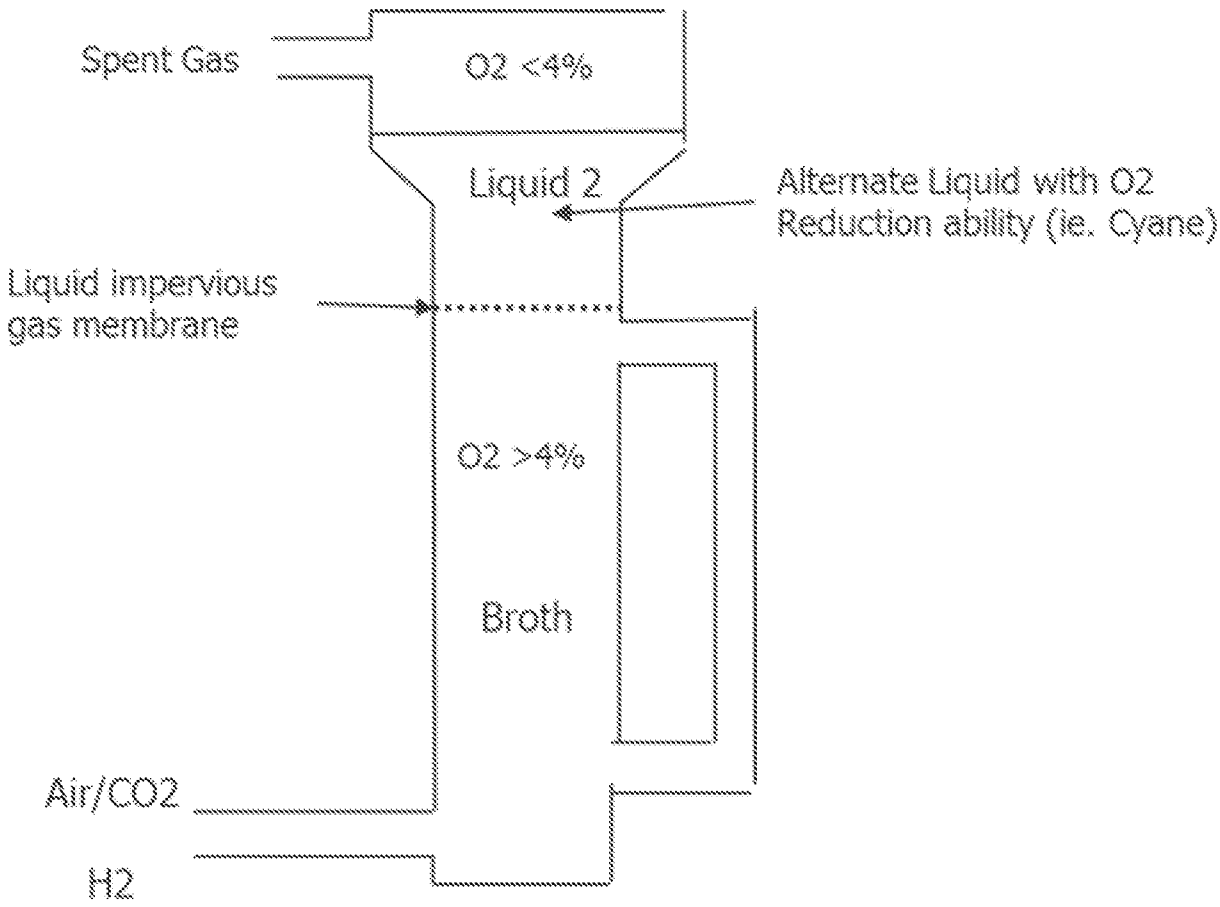
Spent Gas
O2 <4%
Liquid 2
Alternate Liquid with O2
Reduction ability (ie. Cyane)
Liquid impervious
gas membrane
O2 >4%
Broth
Air/CO2
H2

FIG. 3

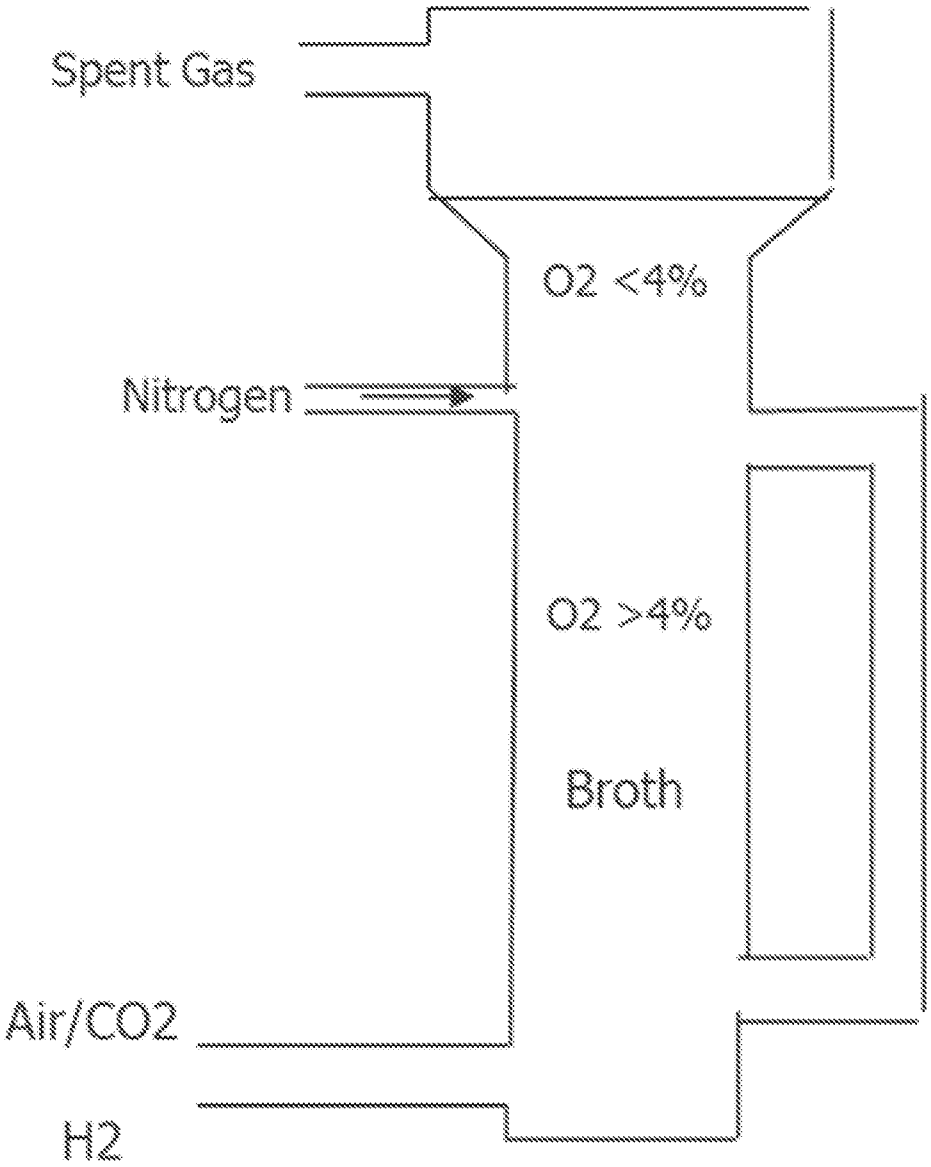

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/025194

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P1/00 C12P5/00
ADD.

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)
C12P C12M

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2017/115855 A1 (AJINOMOTO KK [JP]) 6 July 2017 (2017-07-06) paragraphs [0028], [0080] - [0084]; claims; figures & EP 3 399 015 A1 (AJINOMOTO KK [JP]) 7 November 2018 (2018-11-07) -----	1-14
X	WO 2010/003007 A2 (DANISCO US INC [US]; GOODYEAR TIRE & RUBBER [US] ET AL.) 7 January 2010 (2010-01-07)	1-8,10, 11
Y	paragraphs [0244], [0301], [0302]; claims; example 13 -----	1-14
X	WO 2010/069313 A2 (LARSEN EBBE BUSCH [DK]) 24 June 2010 (2010-06-24) page 26, lines 15-29; claims; figure 2 -----	1-3
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] 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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 August 2019	22/08/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Boeker, Ruth

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/025194

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 995 490 A2 (PRAXAIR TECHNOLOGY INC [US]) 26 April 2000 (2000-04-26) claims	1
A	TANAKA K ET AL: "PRODUCTION OF POLY(D-3-HYDROXYBUTYRATE) FROM CO2, H2, AND O2 BY HIGH CELL DENSITY AUTOTROPIC CULTIVATION OF ALCALIGENES EUTROPHUS", BIOTECHNOLOGY AND BIOENGINEERING, WILEY, vol. 45, no. 3, 5 February 1995 (1995-02-05), pages 268-275, XP000489583, ISSN: 0006-3592, DOI: 10.1002/BIT.260450312 the whole document	1
Y	EP 1 938 892 A1 (MITSUBISHI GAS CHEMICAL CO [JP]) 2 July 2008 (2008-07-02) paragraph [0002]; claims	15-26
X	WO 2008/094282 A1 (UNIV CHICAGO [US]; METS LAURENS [US]) 7 August 2008 (2008-08-07)	15
Y	paragraphs [0041], [0042], [0069], [0070]; claims	15-26

2

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/025194

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

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

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

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

1-26

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

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

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14

   A method for controlling oxygen concentration during aerobic biosynthesis wherein the concentration of the unreacted oxygen in the gaseous phase is reduced by diluting the unreacted oxygen with a dilution agent.

   ---

2. claims: 15-26

   A method for controlling oxygen concentration during aerobic biosynthesis wherein the concentration of the unreacted oxygen in the gaseous phase is reduced by adsorbing or reacting the unreacted oxygen with a solid oxygen catalyst.

   ---

3. claims: 27-38

   A method for controlling oxygen concentration during aerobic biosynthesis wherein the concentration of the unreacted oxygen in the gaseous phase is reduced by absorbing the unreacted oxygen in an oxygen absorbing liquid.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2019/025194

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2011172091 A1	14-07-2011
		WO 2007046449 A1	26-04-2007

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2019/025194

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		EP 2032709 A1	11-03-2009
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