(19) AUSTRALIAN PATENT OFFICE

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

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

17 April 2014 (17.04.2014) WIPO | PCT

WO 2014/058761 A1 **17 April 2014 (17.04.2014)**

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- **(26) Publication Language:** English
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- **(71)** Applicant: **CALYSTA** ENERGY, **LLC [US/US];** 1140 **UG,** ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, **TJ,**
- **(72)** Inventors: SILVERMAN, Joshua; **829** Dartshire Way, **MC,** MK, MT, **NL, NO,** PL, PT, RO, RS, **SE, SI,** SK, **SM,** Sunnyvale, California 94087 (US). **RESNICK, Sol, M.**; **TR), OAPI (BF, BJ, CF, CG, CI, CO, CI, CO, CI, GO, CI, GU, AR, AR, NE**, SN, TD, TG). 818 Nardo Road, Encinitas, California 92024 (US). RE-GITSKY, Drew; 149 Dore Street, #2, San Francisco, Cali- Declarations under Rule 4.17: fornia 94103 (US).
- (74) Agents: CZARNECKI, Michael, **S.** et al.; Seed Intellectu- *patent (Rule 4.17(ii))* ue, Seattle, Washington 98104-7064 **(US).** *earlier application (Rule 4.17(iii))*
- **(81)** Designated States *(unless otherwise indicated, for every* Published: *kind of national protection available)*: **AE, AG, AL, AM,** $\frac{1}{2}$ *with international search report (Art. 21(3))*

<u> 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 199</u>

(10) International Publication Number

(51) International Patent Classification: AO, AT, AU, AZ, BA, BB, BG, BH, **BN,** BR, BW, BY, *E2, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,* (21) International Application Number:

PCT/US2013/063650

PCT/US2013/063650

PCT/US2013/063650

EZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, (22) International Filing Date: **MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,** $\frac{1}{2}$ October 2013 (07.10.2013) OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, **7** October **2013 (07.10.2013)** OM, PA, PE, **PG,** PH, PL, PT, **QA,** RO, RS, RU, RW, **SA, SC, SD, SE, SG,** SK, **SL, SM, ST, SV,** SY, TH, **TJ,** TM, **(25)** Filing Language: English **TN,** TR, TT, TZ, **UA, UG, US, UZ, VC, VN,** ZA, ZM,

(30) Priority Data: (84) Designated States *(unless otherwise indicated, for every* 61/711,104 **8** October 2012 **(08.10.2012) US** *kind of regional protection available):* ARIPO (BW, **GH, GM,** KE, LR, **LS,** MW, MZ, **NA,** RW, **SD, SL,** SZ, TZ, O'Brien Drive, Suite **A,** Menlo Park, California 94025 TM), European **(AL, AT,** BE, BG, **CH,** CY, CZ, **DE,** DK, **(US). EE, ES,** Fl, FR, GB, GR, HR, **HU, IE, IS,** IT, LT, **LU,** LV,

- as to applicant's entitlement to apply for and be granted a
- al Property Law Group PLLC, Suite 5400, 701 Fifth Aven-
ue, Seattle, Washington 98104-7064 (US).
as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

(54) Title: **GAS-FED FERMENTATION SYSTEMS**

(57) Abstract: **A** fermenter can have at least one hollow fluid conduit disposed at least partially within a vessel. An external circum ference of the hollow fluid conduit and an interior circumference of the vessel can define a downward flow path through which a multi-phase mixture including a liquid media and compressed gas substrate bubbles flows. An interior circumference of the hollow quid can flow through the upward flow path and exit the fermenter. Cooling may be provided in the hollow fluid conduit or the ves sel. One or more backpressor generators can be used to maintain a backpressure on the fermenter. One or more fluid movers can be used to variously create an induced and/or forced flow in the downward and upward flow paths.

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GAS-FED FERMENTATION SYSTEMS

BACKGROUND

Technical Field

This invention is generally related to vessels, systems and **5** processes useful in fermentation and, in particular, fermentation systems using a gaseous substrate.

Description of the Related Art

With the ever increasing depletion of fossil fuel deposits, the increasing production of greenhouse gases and recent concerns about climate

- **10** change, substituting biofuels (e.g., ethanol, biodiesel) for fossil fuels has become an industrial focus. But, biofuels generated to date have their own difficulties and concerns. First generation biofuels are derived from plants (e.g., starch; cane sugar; and corn, rapeseed, soybean, palm, and other vegetable oils), but these fuel crops compete with crops grown for human and animal
- **15** consumption. The amount of globally available farm land is insufficient to satisfy the increasing needs for both food and fuel. To reduce the demand placed upon food producers for biofuel compatible grains, second generation biofuels using alternative biological material such as cellulose or algae are under development. But, technical difficulties in production, along with the high
- 20 cost of production, have not made second generation biofuels any more cost effective or accessible.

Third or next generation biofuels are made using alternative, non food based, carbon feedstocks. As part of this effort, the use of alternative, non-biological based, feedstocks in the production of higher hydrocarbon

- **25** compounds including fuels, lubricants, and plastics is gaining ever-increasing momentum. Such feedstocks may include one or more carbon containing compounds or mixtures of carbon containing and non-carbon containing compounds that include, among others, methane and syngas. Methane, for example, is a relatively abundant, naturally occurring and found in many
- **30** locations throughout the world. Methane is also produced during many biological decay processes, and thus may be captured from waste treatment and landfill facilities. For its relative abundance, methane is a potent greenhouse gas, having 23x the relative greenhouse gas contribution **of C02.**

Historically, methane has been viewed as a somewhat valuable byproduct that is difficult to convert to higher value products or to transport to the marketplace from remote or stranded locations such as remote gas fields or off-shore production platforms. Methane from such sources, as well as the methane

- **5** produced **by** biological decomposition processes occurring at sewage treatment facilities and landfills, is primarily either vented or flared. The ability to economically and efficiently convert methane and similar carbon containing gases to one or more higher value C₂ or higher hydrocarbons would permit producers to take advantage of a relatively abundant, non-biologically
- **10** produced, feedstock while at the same time, providing a significant environmental benefit.

The recent rise in domestic production of methane (from 48 billion cubic feet equivalent per day in **2006** to **65** billion cubic feet equivalent per day in 2012) has driven the cost of natural gas to record lows (from about

- **15** \$14.00/MMBTU in **2006** to about **\$2.50/MMBTU** in 2012). Domestic natural gas is primarily produced **by** hydraulic fracturing ("fracking"), but methane can also be obtained from other sources, such as landfills and sewage. But, methane's volatility makes the transport and/or direct usage of methane as a fuel problematic.
- 20 For these reasons, a strong incentive exists to convert the methane to one or more liquid products, for example motor fuels, to permit easier transport to the point of use or sale. Two main approaches are currently being pursued: liquefaction leading to liquefied natural gas **(LNG)** and chemical conversion to convert gas-to-liquid **(GTL)** (Patel, **2005,** 7th World Congress of
- **25** Chemical Engineering, Glasgow, Scotland, **UK).** The Fischer Tropsch (F-T) process is currently the most prevalent approach for converting large quantities of methane to higher-order hydrocarbons (Patel, **2005).** Note that the F-T process takes syngas as an input which is produced from natural gas **by** steam reforming (syngas can also be sourced from coal gasification, **by** high
- **30** temperature reaction with water and oxygen). The F -T process yields petroleum products consistent with today's fuel supply, but suffers from a number of drawbacks, including low yields, poor selectivity (making downstream utilization complex), and requires significant capital expenditure and scale to achieve economical production (Spath and Dayton, December
- **35 2003** NRELITP-510-34929). The massive scale required for an F-T plant (generally in excess of two billion dollars in capital cost [Patel, **2005])** also

represents a significant limitation due to the large amount of methane feedstock required to offset the enormous capital cost of the F-T process. As methane transportation is prohibitively expensive in most cases, such a plant must be co located with a steady, reliable, and cost efficient source of methane, usually in

- **5** the form of a significant methane reservoir or a methane pipeline. An additional cost and scaling factor is the economics of gas-scrubbing technologies (Spath and Dayton, **2003),** since F-T catalysts are quite sensitive to common contaminants found in natural gas that pass unaffected through the syngas conversion process.
- **10** The requirements for ready access to large volumes of a relatively clean methane containing gas, combined with a massive capital investment, currently limit natural gas based F-T plants to successful and economically viable operation in only a few locations world-wide (Spath and Dayton, **2003).** The high minimum processing requirement for a gas-to-liquids process or
- **15** liquified natural gas plant, combined with the high cost of transport, result in smaller methane sources remaining as 'stranded' gas deposits. Such stranded gas can include, but is not limited to, natural gas produced at off-shore oil wells, or methane off-gas from landfills. Due to the current absence of efficient small scale conversion technologies, such stranded gas sources are typically vented
- 20 to atmosphere or flared, as methane accumulation presents a significant safety risk. Gas-to-liquids facilities using the Fischer-Tropsh process have been in operation semi-continuously since **1938.** Several companies are currently investigating introduction of new plants given the current availability and price of methane discussed above. However, despite significant research and
- **25** development over the last **70+** years, the limitations of Fischer-Tropsch technology prevent broad adoption of commercial gas-to-liquids processes. In view of the above limitations, biological fermentation using **C1**

substrates as a carbon source presents an attractive solution to both the current competition between food sources and fermentation for chemicals/fuels,

- **30** as well as the lack of good options for utilization of natural gas. However, fermentation of gaseous substrates such as methane, **CO,** or **C02** presents significant challenges due to the requirement that the carbon substrate must be transferred from the gas phase to an aqueous phase to allow for uptake and metabolism by the C₁ metabolizing non-photosynthetic microorganisms in
- 35 culture. Simultaneously, other gasses such as $0₂$ or H₂ may also be required to be transferred from the gas phase to allow cellular metabolism to progress

(aerobic or anaerobic metabolism, respectively). Waste products (such as **C02** in the case of aerobic metabolism) must be removed rapidly from the reactor to allow for efficient microbial growth. Further, the heat generation from metabolism of C₁ substrates is significant and the system must be cooled **5** continuously to maintain optimal conditions for microbial growth.

Convective mass transfer from the liquid phase to the vapor phase can be described with a mass transfer coefficient. The flux is equal to the product of the mass transfer coefficient, the surface area, and the concentration difference (Flux **= k A AC).**

- **10** The mass transfer coefficient is influenced **by** a variety of factors including the size of the molecule to be transferred, its solubility in the aqueous phase, and the size of the boundary layer between the phases (typically controlled in fermentation systems **by** mixing speed and turbulence). The surface area between the gas and liquid phases in most fermentation systems
- **15** is primarily limited **by** the bubble size of the input gas. Bubble size can be controlled **by** introducing the gas through small pores, as well as increasing shear forces to break apart bubbles and prevent coalescence. The concentration difference can be the concentration difference across the gas phase boundary layer, the concentration difference across the liquid phase
- 20 boundary layer, the concentration difference between the bulk vapor and the vapor which would be in equilibrium with the bulk liquid, or the concentration difference between the bulk liquid and the liquid which would be in equilibrium with the bulk vapor. In most fermentation systems, the concentration difference is controlled **by** the pressure of the gas phase.

25 Conventional fermentation systems (bioreactors) achieve gas mixing **by** one of two methods: stirring or airlift. Stirred fermentors achieve mixing **by** means of stirring blades generally placed centrally in a single large fermentor. The stirrer blades generate turbulence and shear in the liquid while gas bubbles are introduced at the bottom of the fermentor, thus impeding the

- **30** progress of the bubbles as they travel up the fermentor and shearing the gas bubbles to reduce the tendency of the bubbles to coalesce within the fermentor. The advantage of this type of fermentor is the fast, relatively homogeneous mixing and gas bubble dispersion that is possible due to the high speed of the mixing blades. However, this type of fermentor can be difficult to scale-up, as
- **35** the energy requirements to obtain the same rate of mixing and mass transport can be prohibitive as the volume increases. Further, the vigorous mixing

implies a significant heating of the fermentation liquid, and the use of a single large fermentor limits the surface area available for heat exchange cooling.

Airlift fermentors avoid mechanical stirrers **by** incorporating a flow path for the liquid. Airlift fermentors have a downflow and an upflow section which are interconnected at both ends; these sections can either be separate units (referred to as a loop fermentor), or concentric (airlift fermentor). In either case, gasses are supplied at the bottom of the upflow section through a bubble-generating apparatus. The bubbles mix with the liquid, reducing the density of the liquid and causing the gas-liquid mixture to rise through the upflow section. The rising mixture displaces liquid at the top of the reactor, which travels down the downflow section to replace the liquid at the bottom, establishing a circular flow in the fermentor. In order to obtain a long residence time for the gas bubbles in the liquid, airlift fermentors are generally tall and have a limited transverse cross-sectional area. This implies that the gas must be supplied at a relatively high pressure to overcome hydrostatic pressure formed **by** the column of liquid present in the fermentor. In addition, the bubble size increases significantly throughout the fermentor as the pressure decreases with height. The increasing bubble diameter proportionately reduces the rate of mass transfer between the gas bubbles and the liquid phase **by** reducing the ratio of gas bubble volume (proportionate to the cube of the gas bubble radius) to gas bubble area (proportionate to the square of the gas bubble area) through which mass transfer may occur. Flow rates and shear forces in airlift fermentors are significantly lower than in stirred tank fermentors, which also tend to increase bubble coalescence and reduce the efficiency of cooling the fermentor. Finally, separation of the unused and waste gases from the mixture exiting the upflow portion of the fermentor prior to the return of the liquid to the downflow section can be challenging.

OBJECT OF THE INVENTION

It is an object of the present invention to overcome or at least ameliorate one or more of the above disadvantages.

BRIEF SUMMARY

The present disclosure relates to a method and apparatus for efficient mass transfer of gaseous substrates for microbial fermentation. Additionally, this disclosure relates to a method for fermenting gaseous carbon-containing feedstocks using a culture primarily comprising a **C1** metabolizing non-photosynthetic microorganism. This disclosure also relates to a scalable fermentor design for allowing high flux gas-phase to liquid-phase mass transfer in addition to efficient heat exchange and waste gas removal.

In a first aspect, the present invention provides a system to stimulate biological growth, the system comprising:

at least one hollow fluid conduit disposed within an interior space formed **by** a vessel, the at least one hollow fluid conduit permitting a fluid flow therethrough, the at least one hollow fluid conduit including at least one inlet and at least one outlet;

wherein the inlet of the at least one hollow fluid conduit is in fluid communication with the interior space of the vessel to provide a downward flow path bordered **by** an external perimeter of the at least one hollow fluid conduit and **by** an internal perimeter of the vessel and an upward flow path bordered **by** an internal perimeter of the at least one hollow fluid conduit;

at least one gas distributor disposed adjacent the at least one outlet of the at least one hollow fluid conduit and in the downward flow path to introduce one or more gases in at least a portion of the downward flow path;

at least one first fluid mover to provide a downward fluid flow in the downward flow path and an upward fluid flow in the upward flow path; and

a thermal transfer system thermally coupled to at least one of the downward flow path or the upward flow path.

In a second aspect, the present invention provides a method to stimulate biological growth, the method comprising:

dispersing a gas in a liquid media including at least water and one or more nutrients to form a multi-phase mixture comprising gas bubbles dispersed in the liquid media;

flowing at a first velocity and a first pressure the multi-phase mixture in one or more downward flow paths, before flowing the multi-phase mixture in a number of upward flow paths, the one or more downward flow paths formed between an exterior perimeter of a number of hollow fluid conduits and an interior perimeter of a vessel at least partially surrounding the number of hollow fluid conduits;

increasing the pressure of the multi-phase mixture from the first pressure to a second pressure to produce compressed gas bubbles in the multi-phase mixture while contacting the multi-phase mixture with a number of microorganisms existent in the downward flow path to provide a first biomass; and

flowing at a second velocity the multi-phase mixture in a number of upward flow paths, each of the respective number of upward flow paths formed **by** an interior perimeter of each of the number of hollow fluid conduits and maintaining the pressure at or above a third pressure to maintain compressed gas bubbles in the multi-phase mixture while contacting the multi-phase mixture with a number microorganisms existent in the upward flow path to provide a second biomass.

In a third aspect, the present invention provides an apparatus to stimulate biological growth, the apparatus comprising:

a number of hollow fluid conduits disposed at least partially within a vertically arranged vessel to provide a number of downward flow paths bordered **by** an exterior perimeter of each of the number of hollow fluid conduits and an interior perimeter of the vertically arranged vessel;

wherein the vertically arranged vessel includes at least one fluid inlet connection fluidly coupled to at least a portion of the number of downward flow paths;

wherein each of the number of hollow fluid conduits provides a respective number of upward flow paths, each of the upward flow paths bordered **by** an interior perimeter of each of the respective hollow fluid conduits, each of the number of upward flow paths fluidly coupled to at least one discharge connection to remove a multi-phase fluid;

at least one gas distributor disposed in at least a portion of the number of downward flow paths; and

a number of first structures disposed within at least a portion of at least one of: the number of downward flow paths or the upward flow paths, each of the number of first structures (a) to promote biological growth thereupon or **(b)** to promote mass transfer between phases of a multiphase mixture flowing in one of the number of downward flow path or upward flow path in which the number of first structures are disposed.

Such fermentation systems may employ one or more species of microorganism that are preferentially capable of metabolizing C_1 compounds. Such microorganisms include prokaryotes or bacteria, such as Methylomonas, Methylobacter, Methylococcus, Methylosinus, Methylocystis, Methylomicrobium, Methanomonas, Methylophilus, Methylobacillus,

Methylobacterium, Hyphomicrobium, Xanthobacter, Bacillus, Paracoccus, Nocardia, Arthrobacter, Rhodopseudomonas, or Pseudomonas. In some instances, the C₁ metabolizing microorganisms may include methanotrophs, methylotrophs or combinations thereof. Preferred methanotrophs include Methylomonas, Methylobacter, Methylococcus, Methylosinus, Methylocystis, Methylomicrobium, Methanomonas, or combinations thereof. Exemplary methanotrophs include *Methylomonas sp.* 16a **(ATCC** PTA 2402), *Methylosinus trichosporium* (NRRL B-11, **196),** *Methylosinus sporium* (NRRL B-11, **197),** *Methylocystis parvus* (NRRL B-11, *198), Methylomonas methanica* (NRRL *B-5* **11,** *199), Methylomonas alb us* (NRRL B-11 ,200), *Methylobacter capsulatus* (NRRL **B-11** ,20 *1), Methylobacterium organophilum (ATCC 27,886), Methylomonas sp.* **AJ-3670** (FERM P-2400), *Methylomicrobium alcaliphilum, Methylocella silvestris, Methylacidiphilum infernorum, Methylibium petroleiphilum, Methylosinus trichosporium OB3b, Methylococcus capsulatus Bath, Methylomonas sp.* 16a, *Methylomicrobium alcaliphilum* 20Z, or high growth variants thereof. Preferred methylotrophs *include Methylobacterium extorquens, Methylobacterium radiotolerans, Methylobacterium populi, Methylobacterium chloromethanicum, Methylobacterium nodulans,* or combinations thereof.

Microorganisms capable of metabolizing C_1 compounds found in syngas include, but are not limited to Clostridium, Moorella, Pyrococcus, Eubacterium, Desulfobacterium, Carboxydothermus, Acetogenium, Acetobacterium, Acetoanaerobium, Butyribaceterium, Peptostreptococcus, or combinations thereof. Exemplary methylotrophs include *Clostridium autoethanogenum, Clostridium jungdahli, Clostridium ragsdalei, Clostridium carboxydivorans, Butyribacterium methylotrophicum, Clostridium woodii, Clostridium neopropanologen, or* combinations thereof. In some instances, C₁ metabolizing microorganisms are eukaryotes such as yeast, including Candida, Yarrowia, Hansenula, Pichia, Torulopsis, or Rhodotorula.

In other instances, the C_1 metabolizing non-photosynthetic microorganism is an obligate C_1 metabolizing non-photosynthetic microorganism, such as an obligate methanotroph an obligate methylotroph, or combinations thereof. In some instances, the C_1 metabolizing nonphotosynthetic microorganism is a recombinant microorganism comprising a heterologous polynucleotide encoding a fatty acid producing enzyme, a formaldehyde assimilation enzyme, or combinations thereof.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

In the drawings, the sizes and relative positions of elements in the drawings are not necessarily drawn to scale. For example, the various elements and angles are not drawn to scale, and some of these elements are arbitrarily enlarged and positioned to improve drawing legibility. Further, the particular shapes of the elements as drawn are not intended to convey any information regarding the actual shape of the particular elements, and have been selected solely for ease of recognition in the drawings.

Preferred embodiments of the invention will be described hereinafter, **by** way of examples only, with reference to the accompanying drawings, wherein:

Figure 1 shows a perspective view of an example fermentor vessel including a number of hollow fluid conduits disposed within the vessel to create a number of downward flow paths between each of the number of hollow fluid conduits and the surrounding vessel and a number of upward flow paths within each of the number of hollow fluid conduits; the downward and upward flowpaths are fluidly coupled such that at least a portion of the flow in the downward flowpath enters the hollow fluid conduit to provide at least a portion of the flow in the upward flow path, according to one or more illustrated embodiments.

Figure 2 shows a block flow diagram of an example fermentation system including optional cooling subsystem, backpressure subsystem, and separation subsystem that alone or in combination are useful in fermenting a gaseous substrate to provide one or more gaseous or liquid **C2** or higher hydrocarbons, according to one or more illustrated embodiments.

Figure **3** shows a sectional view of an example fermentor including a single hollow fluid conduit disposed within a surrounding vessel to form downward flow path and upward flow path and an externally mounted fluid

mover that forces a flow through both the downward flow path and the upward flow path, according to one or more illustrated embodiments.

Figure 4 shows a sectional view of an example fermentor including a number of hollow fluid conduits at least partially surrounded **by** a

- **5** vessel to form a number of downward flow paths and upward flow paths and an externally mounted fluid mover that forces a flow through both the downward flow path and the upward flow path, according to one or more illustrated embodiments.
- Figure **5** shows a sectional view of an example fermentor **10** including a number of hollow fluid conduits at least partially surrounded **by** a vessel to form a number of downward flow paths and upward flow paths and an externally mounted fluid mover that induces a flow through the downward flow path and forces a flow through the upward flow path, according to one or more illustrated embodiments.
- **15** Figure **6** shows a sectional view of an example fermentor including a number of hollow fluid conduits at least partially surrounded **by** a dished bottom vessel to form a number of downward and upward flow paths and an externally mounted fluid mover that induces a flow through the downward flow path and forces a flow through the upward flow path, according
- 20 to one or more illustrated embodiments.

Figure **7** shows a high level flow diagram of a fermentation method including optional cooling in both the downward and upward flow paths, according to one or more illustrated embodiments.

Figure **8** shows a high level flow diagram of a fermentation **25** method that includes optionally holding the fermentor at an elevated pressure using a backpressure subsystem, according to one or more illustrated embodiments.

Figure **9** shows a high level flow diagram of a fermentation method that includes optionally holding the fermentor at an elevated pressure

30 using a backpressure subsystem, separating the multi-phase mixture removed from the fermentor in an optional separation subsystem, and recycling at least a portion of the separated multi-phase mixture back to the fermentor according to one or more illustrated embodiments.

DETAILED DESCRIPTION

In the following description, certain specific details are set forth in order to provide a thorough understanding of various embodiments. However, one skilled in the art will understand that the invention may be practiced without

- **5** these details. In other instances, structures, standard vessel design details, detailed design parameters of available components such as liquid or gas distributors, pumps, turbines, and similar, details concerning the design and construction of American Society of Mechanical Engineers **(ASME)** pressure vessels, control system theory, specific steps in one or more fermentation
- **10** processes, and the like have not been shown or described in detail to avoid unnecessarily obscuring descriptions of the embodiments. Unless the context requires otherwise, throughout the specification and claims which follow, the word "comprise" and variations thereof, such as, "comprises" and "comprising" are to be construed in an open, inclusive sense, that is, as "including, but not
- **15** limited to." Further, headings provided herein are for convenience only and do not interpret the scope or meaning of the claimed invention.

Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one

- 20 embodiment. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. Also, as used in this specification and the
- **25** appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.
- Fermentors are generally defined as any vessel in which a **30** fermentation process is carried out. Given the vast number of fermentation processes and the wide variety of fermentable substrates, fermentors can range simple continuous stirred tank reactors found in the alcoholic beverage industry to **highly** complex, specialized vessels having gas distribution and internal structures tailored to a particular substrate and/or a particular biological
- **35** species. Fermentors useful in converting carbon containing gases such as methane and syngas (a mixture of CO and H₂) to longer chain gaseous and

liquid hydrocarbons generally disperse a gas substrate containing the **C1** carbon compound within a liquid media containing one or more nutrients to provide a multi-phase mixture. This multi-phase mixture is fed to one or more microbiological colonies that convert a portion of the C₁ carbon compound(s) in

- **5** the gas substrate to more preferred, longer chain, C₂ or higher compounds. The substrate composition, nutrients, and microbiological organisms comprising the colony (i.e., the biomass within the fermentor) can be variously adjusted or tailored to provide a desired final matrix of C_2 or higher compounds which may be present as a liquid, gas, or intracellular material.
- **10** From a mass transfer perspective, gas substrate fermentors present a unique challenge in that the substrate is trapped within a gas bubble and in order for microbiological uptake of the substrate to occur, the gas substrate must first pass from the gas bubble to the microbiological organisms either directly or indirectly via dissolution in the liquid media. Such fermentation
- **15** processes are thus frequently limited **by** the ability of the system to facilitate and/or sustain a desirably high level of mass transfer of the substrate from the gas bubbles to the microbiological organisms within the fermentor. At the least, the rate of mass transfer from the gas bubble to either the surrounding liquid media or to a microbiological organisms is a function of the gas pressure within
- 20 the gas bubble, the volume to surface area ratio of the gas bubble, and the contact time of the gas bubble with the surrounding liquid or microbiological organisms. Increasing the pressure within the gas bubble or increasing the contact time of the gas bubble with the surrounding liquid or microbiological organisms results in a higher effective mass transfer rate between the substrate
- **25** and the microbiological organisms. Decreasing the volume to surface area ratio of the gas bubble (*i.e.*, reducing the diameter of the gas bubbles) results in a higher effective mass transfer rate between the gas bubble and the surrounding liquid. An ideal fermentor would therefore have a large number of relatively small diameter gas bubbles at a relatively high pressure that are held
- **30** in close or intimate contact with the surrounding liquid or microbiological organisms for an extended period of time.

Disclosed herein are a number of fermentation systems, methods, and apparatuses that are capable of providing relatively small diameter, relatively high pressure gas bubbles. Disclosed herein are a number of

35 fermentation systems, methods, and apparatuses capable of providing an extended contact time with the surrounding liquid and/or biological organism(s).

Such fermentation systems, methods, and apparatuses can advantageously provide a **highly** efficient gas substrate fermentation system that has been found particularly useful in converting **C1** compounds to more preferred gaseous, liquid, and intra-cellular C₂ and higher compounds.

5 As used herein, the terms **"C1** substrate" or **"C1** compound" refers to any carbon containing molecule or composition that lacks a carbon-carbon bond. Sample molecules or compositions include methane, methanol, formaldehyde, formic acid or a salt thereof, carbon monoxide, carbon dioxide, syngas, methylamines (e.g., monomethylamine, dimethylamine,

10 trimethylamine), methylthiols, or methylhalogens.

As used herein, the terms "C₁ metabolizing microorganism" or "C₁ metabolizing non-photosynthetic microorganism" refers to any microorganism having the ability to use a single carbon **(C1)** substrate as a source of energy or as its sole source of energy and biomass, and may or may not use other carbon

- **15** substrates (such as sugars and complex carbohydrates) for energy and biomass. For example, a C₁ metabolizing microorganism may oxidize a C₁ substrate, such as methane or methanol. C₁ metabolizing microorganisms include bacteria (such as Methanotrophs and Methylotrophs) and yeast. In at least some instances, a C₁ metabolizing microorganism does not include a
- 20 photosynthetic microorganism, such as algae. In certain embodiments, the **C1** metabolizing microorganism will be an "obligate C₁ metabolizing microorganism," meaning its sole source of energy comprises C₁ substrates and nothing else.

As used herein, the term "methylotrophic bacteria" refers to any **25** bacteria capable of oxidizing organic compounds that do not contain carbon carbon bonds. In certain embodiments, a methylotrophic bacterium may be a methanotroph. For example, "methanotrophic bacteria" refers to any methylotrophic bacteria that have the ability to oxidize methane as its primary source of carbon and energy. Exemplary methanotrophic bacteria include

- **30** Methylomonas, Methylobacter, Methylococcus, Methylosinus, Methylocystis, Methylomicrobium, or Methanomonas. In certain other embodiments, the methylotrophic bacterium is an "obligate methylotrophic bacterium," which refers to bacteria that are limited to the use of C_1 substrates for the generation of energy.
- **35** As used herein, the term **"CO** utilizing bacterium" refers to a bacterium that naturally possesses the ability to oxidize carbon monoxide **(CO)**

as a source of carbon and energy. Carbon monoxide may be utilized from "synthesis gas" or "syngas", a mixture of carbon monoxide and hydrogen produced **by** gasification of any organic feedstock, such as coal, coal oil, natural gas, biomass, and waste organic matter. Carbon monoxide utilizing bacterium

5 does not include bacteria that must be genetically modified for growth on carbon monoxide as its carbon source.

As used herein, the term "syngas" refers to a mixture including at least carbon monoxide (CO) and hydrogen (H₂). In at least some instances, syngas may also include CO₂, methane, and other gases in smaller quantities

10 relative to **CO** and H2 . Syngas may be prepared using any available process, including but not limited to, a water gas shift or coal gasification process.

As used herein, the term "growth" is defined as any increase in cell mass. This may occur through cell division (replication) and the formation of new cells during "balanced growth," or during "unbalanced growth" when

- **15** cellular mass increases due to the accumulation of one or more intracellular or intercellular polymers, such as certain lipids. In the latter case, growth may be manifest as an increase in cell size due to the accumulation of a biopolymer within the cell. During "balanced cell growth," all of the feedstocks (electron donors and electron acceptors) and all of the nutrients are present in the ratios
- 20 required to make all of the macromolecular components of a cell. That is, no feedstock or nutrient limits the synthesis of proteins, complex carbohydrate polymers, fats, or nucleic acids. In contrast, during "unbalanced cell growth," a feedstock or nutrient needed to make one or more of a cell's macromolecules is not present in an amount or ratio required for balanced growth. Accordingly,
- **25** this feedstock or nutrient becomes limiting and is referred to as a "limiting nutrient."

Some cells may still achieve net growth under unbalanced conditions, but the growth is unbalanced and polymers that can be synthesized in the absence of the limiting feedstock or nutrient will accumulate. These

- **30** polymers include lipids or intracellular storage products, such as the polydroxyalkanoates (PHAs), including polyhydroxybutyrate (PHB), polyhdroxyvalerate (PHV), and polyhydroxyhexanoate (PHHx)-glycogen, or secreted materials, such as extracellular polysaccharide. Such oil compositions are useful in the production of bioplastics.
- **35** Sample balanced and unbalanced growth conditions may differ in the nitrogen content in the media. For example, nitrogen constitutes about 12%
	- 12

of dry cell weight, which means that 12 mg/L nitrogen must be supplied (along with a feedstock and other nutrients in the required stoichiometric ratios) to grow **100** mg/L dry cell weight. **If** other feedstock and nutrients are available in the quantities needed to produce **100** mg/L of dry cell weight, but less than 12

- **5** mg/L nitrogen is provided, then unbalanced cell growth may occur, with accumulation of polymers that do not contain nitrogen. **If** nitrogen is subsequently provided, the stored polymer may serve as feedstock for the cell, allowing balanced growth, with replication and production of new cells.
- As used herein, the term "growth cycle" as applied to a cell or **10** microorganism refers to the metabolic cycle through which a cell or microorganism moves in culture conditions. For example, the cycle may include various stages, such as a lag phase, an exponential phase, the end of exponential phase, and a stationary phase.
- As used herein, the term "exponential growth", "exponential phase **15** growth", "log phase" or "log phase growth" refer to the rate at which microorganisms are growing and dividing. For example, during log phase, microorganisms are growing at their maximal rate given their genetic potential, the nature of the medium, and the conditions under which they are grown. Microorganism rate of growth is constant during exponential phase and the
- 20 microorganism divides and doubles in number at regular intervals. Cells that are "actively growing" are those that are growing in log phase. In contrast, "stationary phase" refers to the point in the growth cycle during which cell growth of a culture slows or even ceases.
- As used herein, the term "growth-altering environment" refers to **25** energy, chemicals, or living things that have the capacity to either inhibit cell growth or kill cells. Inhibitory agents may include mutagens, drugs, antibiotics, **UV** light, extreme temperature, **pH,** metabolic byproducts, organic chemicals, inorganic chemicals, bacteria, viruses, or the like.
- As used herein, the term "high growth variant" refers to a **30** organism, microorganism, bacterium, yeast, or cell capable of growth with a **C1** substrate, such as methane or methanol, as the sole carbon and energy source and which possesses an exponential phase growth rate that is faster than the parent, reference or wild-type organism, microorganism, bacterium, yeast, or cell **-** that is, the high growth variant has a faster doubling time and
- **35** consequently a high rate of growth and yield of cell mass per gram of **C1**

substrate metabolized as compared to a parent cell (see, e.g., **U.S.** Patent No. **6,689,601).**

As used herein, the term "biofuel" refers to a fuel at least partially derived from "biomass."

5 As used herein, the term "biomass" refers to a renewable resource having a biological origin.

As used herein, the term "biorefinery" refers to a facility that integrates biomass conversion processes and equipment to produce fuels from biomass.

- **10** As used herein, the term "refinery" refers to an oil refinery, or aspects thereof, at which oil compositions (e.g., biomass, biofuel, or fossil fuels such as crude oil, coal or natural gas) may be processed. Sample processes carried out at such refineries include cracking, transesterification, reforming, distilling, hydroprocessing, isomerization, or any combination thereof.
- **15** As used herein, the terms "recombinant" or "non-natural" refers to an organism microorganism, cell, nucleic acid molecule, or vector that has at least one genetic alternation or has been modified **by** the introduction of a heterologous nucleic acid molecule, or refers to a cell that has been altered such that the expression of an endogenous nucleic acid molecule or gene can
- 20 be controlled. Recombinant also refers to a cell that is derived from a cell having one or more such modifications. For example, recombinant cells may express genes or other nucleic acid molecules that are not found in identical form within the native cell *(i.e., unmodified or wild type cell), or may provide an* altered expression pattern of endogenous genes, such genes that may
- **25** otherwise be over-expressed, under-expressed, minimally expressed, or not expressed at all. In another example, genetic modifications to nucleic acid molecules encoding enzymes or functional fragments thereof can provide biochemical reaction(s) or metabolic pathway capabilities to a recombinant microorganism or cell that is new or altered from its naturally occurring state.
- **30** As used herein, the term "heterologous" nucleic acid molecule, construct or sequence refers to a nucleic acid molecule or portion of a nucleic acid molecule sequence that is not native to a cell in which it is expressed or is a nucleic acid molecule with an altered expression as compared to the native expression levels in similar conditions. For example, a heterologous control
- **35** sequence (e.g., promoter, enhancer) may be used to regulate expression of a gene or a nucleic acid molecule in a way that is different than the gene or a

nucleic acid molecule is normally expressed in nature or culture. Generally, heterologous nucleic acid molecules are not endogenous to the cell or part of the genome in which they are present, and have been added to the cell **by** conjugation, transformation, transfection, electroporation, or the like.

- **5** The systems for fermentation of the instant disclosure may include separate units (e.g., processing units or systems that are disposed in close proximity or adjacent to each other, or not), integrated units, or the system itself may be interconnected and integrated. The systems of this disclosure may use at least one gas phase feedstock, including one or more C₁
- **10** compounds, oxygen, and/or hydrogen. In certain embodiments, the fermentation system uses a C₁ metabolizing microorganism (e.g., a methanotroph such as Methylosinus trichosporium **OB3b,** Methylococcus capsulatus Bath, Methylomonas sp. 16a, Methylomicrobium alcaliphilum 20Z, or high growth variants or combinations thereof) as the primary microorganism
- **15** in the fermentation culture.

A variety of culture methodologies may be used for the microorganism, bacteria and yeast described herein. For example, **C1** metabolizing microorganisms, such as methanotroph or methylotroph bacteria, may be grown **by** batch culture and continuous culture methodologies.

20 Generally cells in log phase are often responsible for the bulk production of a product or intermediate of interest in some systems, whereas stationary or post exponential phase production can be obtained in other systems.

A classical batch culturing method is a closed system in which the media composition is set when the culture is started and is not altered during

- **25** the culture process. That is, media is inoculated at the beginning of the culturing process with one or more microorganisms of choice and then are allowed to grow without adding anything to the system. As used herein, a "batch" culture is in reference to not changing the amount of a particular carbon source initially added, whereas control of factors such as **pH** and oxygen
- **30** concentration can be monitored and altered during the culture. In batch systems, metabolite and biomass compositions of the system change constantly up to the time the culture is terminated. Within batch cultures, cells (e.g., bacteria such as methylotrophs) will generally move from a static lag phase to a high growth logarithmic phase to a stationary phase where growth
- **35** rate is reduced or stopped (and will eventually lead to cell death if conditions do change).

A fed-batch system is a variation on the standard batch system in which a carbon substrate of interest is added in increments as the culture progresses. Fed-batch systems are useful when cell metabolism is likely to be inhibited **by** catabolite repression and when it is desirable to have limited

5 amounts of substrate in the media. Since it is difficult to measure actual substrate concentration in fed-batch systems, an estimate is made based on changes of measurable factors such as **pH,** dissolved oxygen, and the partial pressure of waste gases. Batch and fed-batch culturing methods are common and known in the art (see, e.g., Thomas **D.** Brock, Biotechnology: **A** Textbook of

10 Industrial Microbiology, 2nd **Ed. (1989)** Sinauer Associates, Inc., Sunderland, MA; Deshpande, **1992, Appl.** Biochem. Biotechnol. **36:227).**

Continuous cultures are "open" systems in the sense that defined culture media is continuously added to a bioreactor while an equal amount of used ("conditioned") media is removed simultaneously for processing.

- **15** Continuous cultures generally maintain the cells at a constant high, liquid phase density where cells are primarily in logarithmic growth phase. Alternatively, continuous culture may be practiced with immobilized cells (e.g., biofilm) where carbon and nutrients are continuously added and valuable products, **by** products, and waste products are continuously removed from the cell mass.
- 20 Cell immobilization may be achieved with a wide range of solid supports composed of natural materials, synthetic materials, or a combination thereof.

Continuous or semi-continuous culture allows for the modulation of one or more factors that affect cell growth or end product concentration. For example, one method may maintain a limited nutrient at a fixed rate (e.g.,

- **25** carbon source, nitrogen) and allow all other parameters to change over time. In other embodiments, several factors affecting growth may be continuously altered while cell concentration, as measured **by** media turbidity, is kept constant. The goal of a continuous culture system is to maintain steady state growth conditions while balancing cell loss due to media being drawn off
- **30** against the cell growth rate. Methods of modulating nutrients and growth factors for continuous culture processes and techniques for maximizing the rate of product formation are well known in the art (see Brock, **1992).**

In certain embodiments, culture media includes a carbon substrate as a source of energy for a C₁ metabolizing microorganism. Suitable

35 substrates include **C1** substrates, such as methane, methanol, formaldehyde, formic acid (formate), carbon monoxide, carbon dioxide, methylated amines

(methylamine, dimethylamine, trimethylamine, etc.), methylated thiols, or methyl halogens (bromomethane, chloromethane, iodomethane, dichloromethane, etc.). In certain embodiments, culture media may comprise a single C₁ substrate as the sole carbon source for a C₁ metabolizing microorganism, or

- **5** may comprise a mixture of two or more **C1** substrates (mixed **C1** substrate composition) as multiple carbon sources for a **C1** metabolizing microorganism. Additionally, some C₁ metabolizing organisms are known to utilize non- C_1 substrates, such as sugar, glucosamine or a variety of amino acids for metabolic activity. For example, some Candida species can metabolize alanine
- **10** or oleic acid (Sulter et al., Arch. Microbiol. **153:485-489, 1990).** Methylobacterium extorquens AM1 is capable of growth on a limited number of **C2 , C3 ,** and C4 substrates (Van Dien et al., Microbiol. **149:601-609, 2003).** Alternatively, a C₁ metabolizing microorganism may be a recombinant variant having the ability to utilize alternative carbon substrates. Hence, it is
- **15** contemplated that a carbon source in culture media may comprise a mixture of carbon substrates, with single and multi-carbon compounds, depending on the **C1** metabolizing microorganism selected.

In certain embodiments, the instant disclosure provides a method for making fuel, comprising converting biomass from a culture primarily

- 20 comprising a **C,** metabolizing non-photosynthetic microorganism into an oil composition and refining the oil composition into a fuel. In certain embodiments, the **C,** metabolizing non-photosynthetic microorganism is an obligate **C1** metabolizing non-photosynthetic microorganism, such as an obligate methanotroph or methylotroph. In further embodiments, the **C1**
- **25** metabolizing non-photosynthetic microorganism is a recombinant microorganism comprising a heterologous polynucleotide encoding a fatty acid producing enzyme, a formaldehyde assimilation enzyme, or a combination thereof. In further embodiments, the oil composition is derived or extracted from cell membrane of the C₁ metabolizing non-photosynthetic microorganism, **30** such as a methylotroph or methanotroph.
	- In certain embodiments, the instant disclosure provides a method for making fuel **by** refining an oil composition in a refining unit to produce fuel, wherein the oil composition is derived from a **C,** metabolizing non photosynthetic microorganism, such as a methylotroph or methanotroph. In
- **35** further embodiments, the method further comprises use of a processing unit for extracting the oil composition from the C₁ metabolizing non-photosynthetic
	- **17**

microorganism. In still further embodiments, the method comprises (a) culturing **C1** metabolizing bacteria in the presence of a feedstock comprising a **C1** substrate in a controlled culturing unit, wherein the cultured bacteria produces an oil composition; **(b)** extracting the oil composition from the cultured

- **5** bacteria in a processing unit; and (c) refining the extracted oil composition in a refining unit to produce fuel. In certain embodiments, the feedstock **C1** substrate is methane, methanol, formaldehyde, formic acid, carbon monoxide, carbon dioxide, a methylamine, a methylthiol, or a methylhalogen.
- In certain embodiments, the instant disclosure provides a method **10** for making natural products, such as ethanol, acetate, butanol, single-cell protein, sugars, or other metabolites or cellular products wherein the natural product is derived from a **Ci** metabolizing non-photosynthetic microorganism, such as a methylotroph or methanotroph.
- In further embodiments, the method further comprises use of a 15 processing unit for extracting the natural product from the C₁ metabolizing nonphotosynthetic microorganism.

In still further embodiments, the method comprises (a) culturing C₁ metabolizing bacteria in the presence of a feedstock comprising a C₁ substrate in a controlled culturing unit, wherein the cultured bacteria produce a natural

- 20 product; **(b)** extracting the natural product from the cultured bacteria in a processing unit; and (c) refining the natural product to produce a commercial product. In certain embodiments, the feedstock C₁ substrate is methane, methanol, formaldehyde, formic acid, carbon monoxide, carbon dioxide, a methylamine, a methylthiol, or a methylhalogen.
- **25** In certain embodiments, the instant disclosure provides a method for making natural or non-natural products, such as ethanol, acetate, butanol, isoprene, propylene, farnesene, enzymes, or other metabolites or cellular products wherein the product is derived from a genetically engineered **C1** metabolizing non-photosynthetic microorganism, such as a methylotroph or
- **30** methanotroph which has been transformed with a heterologous nucleotide sequence. In further embodiments, the method further comprises use of a processing unit for extracting the product from the genetically engineered **C1** metabolizing non-photosynthetic microorganism. In still further embodiments, the method comprises (a) culturing genetically engineered C_1 metabolizing
- **35** bacteria in the presence of a feedstock comprising a **C1** substrate in a controlled culturing unit, wherein the cultured bacteria produce a natural product; **(b)**

extracting the natural product from the cultured bacteria in a processing unit; and (c) refining the natural product to produce a commercial product. In certain embodiments, the feedstock C₁ substrate is methane, methanol, formaldehyde, formic acid, carbon monoxide, carbon dioxide, a methylamine, a methylthiol, or

5 a methylhalogen.

In certain embodiments, the instant disclosure provides a method for making natural or non-natural products, such as ethanol, acetate, butanol, isoprene, propylene, farnesene, enzymes, or other metabolites or cellular products wherein the product is derived from a non- C_1 metabolizing

- **10** microorganism, such as Escherichia coli, Saccaromyces cerevisiae, or other common production microorganism. In certain embodiments, the feedstock substrate is glucose, sucrose, glycerol, cellulose or other multicarbon feedstocks.
- Figure **1** shows an example fermentor **100** including a number of **15** hollow fluid conduits 102 (only one shown in Figure **1** for clarity) disposed within an interior space **106** formed **by** a vessel **108.** The vessel **108** can include one or more walls **110,** a bottom 124 and an optional top **126** that partially or completely encloses the hollow fluid conduit 102, the vessel **108,** or both. At least a portion of the space existent between the number of hollow fluid
- 20 conduits 102 and the vessel **108** provides one or more downward flow paths 114 through which a downward flow **116** may occur. At least a portion of the space existent within each of the number of hollow fluid conduits 102 provides one or more upward flow paths **118** through which an upward flow 120 may occur. One or more liquid distributors **130** may be disposed in, on, or about the
- **25** vessel **108** to introduce one or more liquids to the downward flow path **114.** One or more gas distributors **132** may be disposed in, on, or about the vessel **108** to introduce one or more gases or gas substrates to the downward flow path **114.** The positioning of the hollow fluid conduit 102 within the vessel **108** advantageously allows for a more accurate and a more consistent scaling than
- **30** either loop or airlift fermentor designs, since the volume of the fermentor **100** is scaled based upon the radius or cross-sectional profile rather than length.

The addition of one or more substrate gases via the one or more gas distributors **132** in some instances, promotes the formation of finely divided substrate gas bubbles to the liquid within the downward flow path 114. The

35 combination of the liquid and the finely divided, dispersed gas or gas substrate bubbles creates a multi-phase mixture having a generally a downward flow **116**

in the downward flow path **114.** The downward flow **116** includes a significant number of entrained gas or substrate gas bubbles dispersed within the multi phase mixture liquid. The difference in density between the gas bubbles and the liquid in the multi-phase mixture causes the gas or gas substrate bubbles to

- **5** tend to rise upward. However, **by** maintaining the superficial velocity of the downward flow **116** in the downward flow path **114** at a rate greater than the gas bubble rise rate, gas substrate bubbles present in the multiphase mixture in the downward flow path **114** will travel at a reduced velocity in a generally downward direction. The net velocity of the gas substrate bubbles in the
- **10** downward flow path 114 is the superficial velocity of the multiphase fluid in the downward flow path 114 minus the rise rate of the gas substrate bubbles. Since the gas substrate bubbles travel through the downward flow path **114** at a velocity less than the comparable rise rate in an airlift fermentor, contact time between the gas substrate bubbles and microbiological organisms existent
- **15** within the downward flow path 114 is advantageously increased over airlift type fermentors. Although only one liquid distributor **130** and one gas distributor **132** are shown in Figure **1** for clarity, any number of additional liquid distributors, gas distributors, or combinations thereof may be added at regular or irregular intervals within the downward flow path 114.
- 20 The multi-phase mixture in the downward flow path 114 enters 122 the upward flow path **118** via one or more fluid conduits, openings, orifices, apertures, or gaps fluidly connecting or fluidly coupling the number of hollow fluid conduits 102 (i.e., the upward flow path **118)** to the interior space **106** of the vessel **108** existent between the vessel wall **110** and the number of hollow
- **25** fluid conduits 102 (i.e., the downward flow path). In one instance, the multi phase mixture in the downward flow path **114** can enter the hollow fluid conduit 102 via a gap between the hollow fluid conduit and the bottom 124 of the vessel **108.** In at least some instances, the bottom 124 of the vessel **108** can be shaped, formed, or configured to promote the accumulation of biological
- **30** material (i.e., "biosolids" or "biomass") at a desired location within the vessel **108.** For example, the bottom 124 can be conically shaped, dished, or sloped such that biomass falling to the bottom of the fermentor **100** preferentially collects in one or more predetermined locations. In such instances, each of the number of hollow fluid conduits 102 may have different lengths in order to
- **35** maintain a defined desired or preferred gap or spacing between the inlet of

each of the number of hollow fluid conduits 102 and the bottom 124 of the vessel 108.

Having entered the upward flow path **118,** the multi-phase mixture creates an upward flow 120 therein. Both the fluid and the gas bubbles in the **5** upward flow path **118** will travel in a generally upward direction. The rise rate of the gas bubbles within the upward flow path **118** is equal to the velocity of the fluid plus the rise rate of the gas bubbles within the fluid. In at least some instances, any number of additional liquid distributors **130,** gas distributors **132,** or combinations thereof may be added at regular or irregular intervals within the

10 upward flow path **118.**

The multi-phase mixture **136** flows from the upward flow path **118** and exits the fermentor **100** via one or more multi-phase mixture discharge connections **138.** In at least some instances, one or more multi-phase mixture discharge connections **138** may fluidly coupled to each of the upward flow

- **15** paths **118** provided **by** the number of hollow fluid conduits 102. In some instances, the one or more multi-phase mixture discharge connections **138** can include one or more flanged or threaded connections. In some instances, the one or more multi-phase mixture discharge connections **138** can include one or more quick disconnect or similar easily sterilizable fluid couplings or
- 20 connections.

In some instances, thermal energy (e.g., in the form of sensible heat) may be generated or liberated **by** the fermentation process or processes occurring within the fermentor **100.** Left uncontrolled, sufficient thermal energy can build within the fermentor **100** to adversely affect the growth or metabolism

- **25** of the microbiological organisms within the fermentor **100.** In some instances, uncontrolled thermal energy increases can result in the death of all or a portion of the microbiological organisms within the fermenter. To remove at least a portion of the thermal energy from the fermentor **100,** one or more thermal transfer surfaces **128** may be disposed on the interior or exterior of the hollow
- **30** fluid conduit 102, one or more thermal transfer surfaces 140 may be disposed on the interior or exterior of the vessel **106,** or such thermal transfer surfaces **128,** 140 may be disposed on any combination of surfaces in fluid and thermal contact with either or both the downward flow path **114** and the upward flow path **118.**
- **35** The fermentor **100** can include any number of hollow fluid conduits 102 disposed wholly or partially in the interior space **106** formed **by** a

vessel **108.** Each of the number of hollow fluid conduits 102 can include any size, shape, or configuration closed fluid channel having a constant or variable cross-sectional profile and a constant or variable wall thickness. Each of the hollow fluid conduits 102 includes at least one opening or inlet that permits the

- **5** entry of the multi-phase mixture into the upward flow path **118** on the inside of each of the number of hollow fluid conduits 102. Each of the number of hollow fluid conduits 102 includes at least one opening or outlet that permits the exit of the multi-phase mixture **136** from the upward flow path **118.** The vessel **108** can have any size, shape, or configuration having one or more walls **110** that
- **10** form or otherwise define the interior space **106.** The interior perimeter formed **by** the one or more walls **110** provides the transverse cross-sectional profile and the transverse cross-sectional area of the vessel **108.** The one or more walls **110** also form the interior perimeter **150** of the vessel **108.**
- Each of the hollow fluid conduits 102 includes an interior **15** perimeter **152,** at least a portion of which is in fluid contact with the upward flow path **118,** and an exterior perimeter 154, at least a portion of which is in fluid contact with the downward flow path **114.** The downward flow path **114** is bounded **by** the exterior perimeter 154 of the hollow fluid conduit 102 and the interior perimeter **150** of the vessel **108.** The upward flow path 120 is bounded
- 20 **by** the interior perimeter 154 of each of the respective number of hollow fluid conduits 102. Each of the hollow fluid conduits 102 is fluidly coupled to the interior space **106** within the vessel **108,** thereby permitting a free flow of the multi-phase mixture from the downward flow path **114** to the upward flow path **118.**
- **25** In at least some instances, the aggregate transverse cross sectional area of the number of hollow fluid conduits 102 is about **90%** or less of the transverse cross-sectional area of the vessel **108;** about **75%** or less of the transverse cross-sectional area of the vessel **108;** about **50%** or less of the transverse cross-sectional area of the vessel **108;** about **25%** or less of the
- **30** transverse cross-sectional area of the vessel **108;** about **15%** or less of the transverse cross-sectional area of the vessel **108;** or about **10%** or less of the transverse cross-sectional area of the vessel **108.**

One or more liquids may be introduced to the downward flow path **114** using the one or more liquid distributors **130** that are positioned in or fluidly **35** coupled to the downward flow path 114. Such liquids can include any liquid media capable of supporting or transporting dissolved or suspended

sustenance or nutrients to the microbiological organisms forming the biomass within the fermentor **100.** One or more gases, substrate gases, or combinations thereof may be introduced to the downward flow path 114 via the one or more gas distributors **132** that are positioned in or fluidly coupled to the

- **5** downward flow path 114. Such gases can include a single gas or a combination of gases capable of supporting or providing sustenance or nutrients to the biological organisms within the fermentor **100.** In at least some instances, such gases may include one or more inert gases, for example nitrogen. In at least some instances, a plurality of gases, a plurality of substrate
- **10** gases, or some combination thereof may be introduced separately to the downward flow path 114 to advantageously reduce or even preclude the formation of explosive gas mixtures outside of the fermentor **100.** For example, where an inflammable C_1 compound (e.g., methane) is used to provide at least a portion of a gaseous substrate for the biological organisms within the
- **15** fermentor **100** and where air is used to provide oxygen to the biological organisms within the fermentor **100,** the gas containing the inflammable **C1** compound may be introduced using a first gas distributor 132a and the air introduced using a second, physically distinct or separate, gas distributor **132b** to avoid mixing of the C₁ compound and the air outside of the fermentor 100.
- 20 The introduction of one or more liquids and one or more gases to the downward flow path **114** creates a downward flow **116** of a multi-phase fluid within the downward flow path 114. Biological growth occurring within the downward flow path 114 can absorb oxygen, nutrients and C₁ compounds from the downward flow **116** of multi-phase fluid in the downward flow path 114.

25 The contemporaneous introduction of one or more inflammable **C1** compounds and an oxygen containing gas into the fermentor **100** may result in the formation of flammable or explosive gas mixtures within the fermentor **100.** To reduce the possibility of such an occurrence, the downward flow path 114 and the upward flow path **118** may be of a physical shape or configuration such

- **30** that both remain "fluid full" when liquid is introduced to the fermentor **100** via the one or more liquid distributors **130** (i.e., gas accumulation points within the downward flow path 114 and the upward flow path **118** are minimized, or more preferably, eliminated). In at least some instances, one or more gas relief devices or liquid/gas separation devices (not shown in Figure **1)** may be fluidly
- **35** coupled to the downward flow path **114,** the upward flow path **118** or both to remove accumulated gases from the fermentor **100.** Such removed gases may

be partially or completely recovered and partially or completely recycled to the fermentor **100** or may be flared or otherwise safely disposed of.

Each of the number of hollow fluid conduits 102 can have the same or different transverse cross-sectional profile. Example hollow fluid

- **5** conduit 102 transverse cross-sectional profiles include, but are not limited to, a circular transverse cross-sectional profile, a rectangular or square transverse cross-sectional profile, or a triangular transverse cross-sectional profile (i.e., a profile taken transverse to the longitudinal axis of the hollow fluid conduit 102). In some instances, more than one type of hollow fluid conduit 102 may be used
- **10** within a single fermentor **100.** For example, a portion of the number of hollow fluid conduits 102 in the fermentor **100** may have a circular transverse cross sectional profile, while the remaining portion of the hollow fluid conduits 102 have a square transverse cross-sectional profile.
- **All** or a portion of the hollow fluid conduits 102 can include one or **15** more embossed or debossed surface features useful in supporting, promoting, or otherwise fostering the growth of biological organisms. **All** or a portion of the hollow fluid conduits 102 can include one or more embossed or debossed surface features useful in promoting or otherwise enhancing turbulence or heat transfer to the respective thermal transfer surface(s) **128,** 140 in the downward
- 20 flow path **114,** the upward flow path 120, or both. **All** or a portion of the hollow fluid conduits 102 can include one or more baffles to increase turbulence within the upward flow path **118,** such increases in turbulence can advantageously improve mass transfer within the upward flow path **118** as well as increase the residence time of gas substrate bubbles present in the upward flow path **118.**
- **25** In some instances, the longitudinal axis of each of the number of hollow fluid conduits 102 is aligned parallel to the longitudinal axis of every other hollow fluid conduit 102 disposed in the vessel **108.** In some instances, the longitudinal axis of each of the number of hollow fluid conduits 102 is parallel to the longitudinal axis of the vessel **108.** In some instances, the
- **30** longitudinal axis of each of all or a portion of the number of hollow fluid conduits 102 is parallel to and coaxially aligned with the longitudinal axis of the vessel **108.**

Each of the hollow fluid conduits 102 can have the same or a different length. In some instances, at least a portion of the number of hollow **35** fluid conduits 102 may extend to or even beyond the bottom 124 of the vessel **108.** In such instances, the downward flow path 114 may be fluidly coupled to

the upward flow path **118** via one or more external fluid conduits, for example via one or more piping networks positioned external to the vessel **108.** Each of the hollow fluid conduits 102 can have a length of from about **6** inches to about 240 inches; from about 12 inches to about **192** inches; or from about 24 inches

5 to about 144 inches.

Each of the number of hollow fluid conduits 102 may be a metallic, non-metallic, or composite structure. For example, each of the hollow fluid conduits 102 may include one or more metallic materials such as 304, 304L, **316,** or **316L** stainless steels. In some instances, one or more coatings,

- **10** layers, overlays, inserts, or other materials may be deposited on, applied to, joined with, or formed integral to all or a portion of each of the number of hollow fluid conduits 120 to beneficially or detrimentally affect the ability for microbiological organisms to attach thereto or to grow thereupon. For example, a coating inhibiting the growth or attachment of microbiological organisms may
- **15** be deposited on or formed integral with the portion of the hollow fluid conduits 102 that are thermally conductively coupled to the thermal transfer surfaces **128.** In another example, a coating that promotes the growth or attachment of biological organisms may be deposited on or formed integral with the hollow fluid conduits 102 in areas where removal of accumulated biomass is more
- 20 easily accomplished.

In at least some instances, each of the hollow fluid conduits 102 includes a substantially rectangular or square hollow member having an aperture extending along the longitudinal axis of the conduit. In other instances, each of the hollow fluid conduits 102 includes a substantially

- **25** cylindrical hollow member having an aperture extending along the longitudinal axis of the conduit. The inside and outside diameters of such square or cylindrical hollow fluid conduits 102 may be continuous or constant along the axial length of the conduit or may vary along the axial length of the conduit. For example, the inside and outside diameters of a cylindrical hollow fluid conduit
- **30** 102 may increase co-currently along the upward flow path **118** such that the fluid velocity along the upward flow path decreases from the inlet to the outlet of the hollow fluid conduit 102. Cylindrical hollow fluid conduits 102 can have an inside diameter of from about 2 inches to about 240 inches; from about 4 includes to about **192** inches; from about **6** inches to about 144 inches; from
- **35** about **8** inches to about 120 inches; or from about 12 inches to about **96** inches. Rectangular or square hollow fluid conduits 102 can have a diagonal of from

about 2 inches to about 240 inches; from about 4 includes to about **192** inches; from about **6** inches to about 144 inches; from about **8** inches to about 120 inches; or from about 12 inches to about **96** inches.

In at least some instances, the construction of the hollow fluid **5** conduits 102, vessel **108,** or both, may include features that facilitate sterilization of all or a portion of the process contact surfaces. Such sterilization can be accomplished for example using steam sterilization, ultraviolet sterilization, chemical sterilization, or combinations thereof. In at least some instances, one or more non-metallic materials or one or more non-metallic

- **10** coatings may be used within all or a portion of the interior or exterior of some or all of the number of hollow fluid conduits 102. The use of such non-metallic materials may advantageously provide, for example, sterilizable surfaces that are capable of supporting or promoting biological growth.
- **All** or a portion of the number of hollow fluid conduits 102 may **15** optionally include one or more thermal energy transfer surfaces **128** useful in limiting heat buildup within either or both the downward flow path **114** or the upward flow path 120. The thermal energy transfer surfaces **128** can include one or more conduits or reservoirs disposed along the interior perimeter **152,** the exterior perimeter 154, or both the interior and exterior perimeter of the
- 20 hollow fluid conduit 102. The one or more conduits or reservoirs can include any device or system through which one or more thermal transfer media can flow. In at least some instances, the one or more thermal energy transfer surfaces **128** can be formed integrally with the hollow fluid conduit 102, forming at least a portion of the conduit. In one example, the thermal energy transfer
- **25** surface **128** can include one or more reservoirs through which a thermal transfer fluid can flow or circulate, and which is thermally conductively coupled to at least a portion of the hollow fluid conduit 102. In another example, the thermal energy transfer surface **128** can include one or more pipe or tubing coils or similar structures through which a thermal transfer fluid can flow or
- **30** circulate, and which is thermally conductively coupled to at least a portion of the hollow fluid conduit 102. The thermal transfer media can include any material, including gases or liquids, that are capable of either providing thermal energy to or removing thermal energy from either or both the downward flow path **114** or the upward flow path **118.** Example thermal transfer media include, but are not
- **35** limited to, cooling water, chilled water, and steam.

Each of the hollow fluid conduits 102 can be permanently or detachably attached to the vessel **108.** In at least some instances, the hollow fluid conduit multi-phase mixture discharge fluid connections **138** may be attached or otherwise affixed to the top **126** of the vessel **108** to allow the multi

5 phase mixture **136** flowing through the upward flow path **118** to exit each of the number of hollow fluid conduits 102.

The height of the vessel **108** can be the same as or different from the length of all or a portion of the number of hollow fluid conduits 102. Where the height of the vessel **108** is less than the length of all or a portion of the

- **10** number of hollow fluid conduits 102, all or a portion of the hollow fluid conduits 102 may project from the bottom 124 of the vessel **108,** top **126** of the vessel **108,** or both the top and the bottom of the vessel **108.** The vessel **108** can have a height of from about **6** inches to about 240 inches; from about 12 inches to about **192** inches; or from about 24 inches to about 144 inches.
- **15** The vessel **108** can have a regular or an irregular transverse cross-sectional profile (i.e., a profile taken transverse to the longitudinal axis of the vessel **108).** Example vessel **108** transverse cross-sectional profiles include, but are not limited to, a circular transverse cross-sectional profile, a square transverse cross-sectional profile, or a triangular transverse cross
- 20 sectional profile. For example the transverse cross sectional profile of the vessel **108** may decrease co-current with the downward flow path **114** such that the fluid velocity and pressure of the multi-phase mixture increases along the downward flow path **114.**
- **All** or a portion of the wall(s) **110,** bottom 124, or top **126** of the **25** vessel **108** may optionally include one or more thermal energy transfer surfaces 140 useful in limiting heat buildup within the downward flow path 114. The thermal energy transfer surfaces 140 can include one or more conduits or reservoirs thermally conductively coupled to at least a portion of the downward flow paths 114 within vessel **108** and through which one or more thermal
- **30** transfer media can flow. For example, the thermal energy transfer surfaces 140 can include one or more reservoirs through which a thermal transfer fluid can flow or circulate. In another example, the thermal energy transfer surfaces 140 can include one or more pipe or tubing coils or similar structures through which a thermal transfer fluid can flow or circulate. The thermal transfer media can
- **35** include any material, including gases or liquids, capable of either providing thermal energy to or removing thermal energy from the downward flow path

114. Example thermal transfer media include, but are not limited to, cooling water, chilled water, and steam.

In at least some instances, the vessel **106** may be a metallic, non metallic, or composite structures. Example metallic structures include vessel

- **5** walls, top and bottom surfaces fabricated from materials such as 304, 304L, **316,** or **316L** stainless steels. Example non-metallic structures include, but are not limited to, fiberglass, fiberglass reinforced plastic (FRP), polyethylene, and the like. Example composite structures include, but are not limited to, metal reinforced FRP and the like. In some instances, one or more coatings or other
- **10** materials may be formed integral with or applied to the vessel **108** to variously affect the ability for microbiological organisms to attach thereto. For example, a coating inhibiting the growth or attachment of microbiological organisms may be deposited on or formed integral with the vessel **108** in areas occupied **by** thermal transfer surfaces 140. In another example, a coating that promotes the
- **15** growth or attachment of microbiological organisms may be deposited on or formed integral with the vessel **108** in areas where removal of accumulated biomass is more easily accomplished.

One or more liquid distributors **130** are disposed either within the downward flow path 114, within the upward flow path **118,** fluidly coupled to the 20 downward flow path 114, fluidly coupled to the upward flow path 120, or combinations thereof. The one or more liquid distributors **130** can include any device, structure or system capable of distributing fluid in any desired pattern within the selected flow path. In some instances, all or a portion of the one or

more liquid distributors **130** can include a pipe or distribution plate containing a

- **25** number of orifices or apertures through which fluid enters the respective flow path. In some instances, all or a portion of the one or more liquid distributors **130** can include a fluid connection such as a nozzle or coupling disposed at an appropriate location on the vessel **108.** In at least some instances, liquid distributors **130** may be positioned at multiple locations within either or both the
- **30** downward flow path 114, the upward flow path 120, or both. The use of liquid distributors **130** positioned at multiple locations within the downward flow path **114** and/or the upward flow path 120 advantageously permits the introduction of liquids containing one or more components useful in promoting one or more desirable qualities in the accumulated biomass. For example, a liquid
- **35** containing one or more growth promoting components may be introduced using a first liquid distributor 130a positioned in the downward flow path 114 and a

liquid containing one or more components that increase the production of one or more desired compounds may be introduced using a second liquid distributor **1 30b** positioned at a later point in the downward flow path **114** or in the upward flow path 120.

- **5** The one or more liquid distributors **130** are sized or selected based upon expected flow, operating temperature, operating pressure, and allowable pressure drop. The liquid distributors **130** may include a number of orifices through which liquid in the distributor exits, such orifices can be sized and positioned based upon a defined flow range and an allowable pressure
- **10** drop range. In some instances, liquid exiting the one or more liquid distributors **130** may flow through one or more dispersion or mixing devices that promote dispersion of or mixing between the liquid exiting the distributor **130** and the multi-phase mixture flowing through the respective flow path.
- One or more gas distributors **132** are disposed either within or in **15** fluid contact with the downward flow path 114, the upward flow path 120, or both. The one or more gas distributors **132** can include any device, structure or system capable of distributing in any defined pattern one or more gases having a defined physical gas bubble size or shape within the selected flow path. In some instances, all or a portion of the one or more gas distributors **132** can
- 20 include a pipe or distribution plate containing a number of orifices or apertures through which a gas, a gas substrate, or combinations thereof are introduced to the flow path. In some instances, all or a portion of the one or more gas distributors **132** can include a sintered metal or porous ceramic distribution plate capable of producing a multitude of fine gas bubbles within the selected
- **25** flow path. In at least some instances, gas distributors **132** may be positioned at multiple locations within either or both the downward flow path 114, the upward flow path **118,** or both.

Gas substrate bubbles may be introduced at a location near the liquid distributor **130** (i.e., at the start of the downward flow path **114).** The

- **30** introduction of gas substrate bubbles at a location near the liquid distributor **130** advantageously permits pushing of the gas substrate bubbles along the downward flow path **114 by** the flow of the multi-phase mixture through the downward flow path 114. Passing the gas substrate bubbles downward along the downward flow path 114 may advantageously improve mass transfer, as
- **35** the gas substrate bubble size will tend to decrease with the increase in pressure along the downward flow path 114 attributable to the increasing

hydrostatic head. The gas substrate bubbles present in the downward flow **¹¹⁶** of the multi-phase mixture will tend to get smaller as they travel along the downward flow path **114** and through the fermentor **100.** Advantageously, shear forces and heat generation within the fermentor **100** are reduced since a

5 conventional agitator is not needed to disperse the gas substrate bubbles or to maintain the dispersion of the gas substrate bubbles present in the multi-phase mixture within the fermentor **100.**

The use of gas distributors **132** positioned at multiple locations within the downward flow path 114 and/or the upward flow path 120

- **10** advantageously permits the introduction of gaseous substrates containing one or more components useful in promoting one or more desirable qualities in the accumulated biomass. For example, a gas substrate containing one or more growth promoting components may be introduced using a first gas distributor 132a positioned in the downward flow path 114 and a gas substrate containing
- **15** one or more components that increase the production of one or more desired compounds may be introduced using a second gas distributor **132b** positioned at a later point in the downward flow path 114 or in the upward flow path 120. In at least some instances, the gas distributor **132** can provide gas substrate bubbles having a diameter of from about **0.01** inches to about **1** inch; from
- 20 about **0.05** inches to about **0.75** inches; from about **0.075** inches to about **0.75** inches; or from about **0.1** inches to about **0.5** inches.

Figure 2 shows an example fermentation system 200 that includes a fermentor **100** along with an optional backpressure subsystem **230,** a separation subsystem **250,** and an optional thermal subsystem **270.** Although

- **25** shown as an integrated system 200, the optional subsystems may be installed or otherwise combined with the fermentor **100** either individually or in any combination. One or more liquids 202 and one or more gas substrates 204 are introduced to the fermentor **100** to form a multi-phase mixture therein that travels through the fermentor **100** via the downward flow path(s) **114** and the
- **30** upward flow path(s) **118.** After passage through the fermentor **100,** the multi phase fluid may contain one or more compounds produced **by** the biological organisms within the fermentor **100,** unconsumed nutrients and other compounds in the liquid within the multi-phase mixture, unconsumed gases in the gas bubbles within the multi-phase mixture, and microbiological organisms
- **35** in the form of biosolids. Excess microbiological organisms may be removed from the fermentor **100** as biomass either intermittently or continuously via at

least one biomass removal fluid connection **208.** Biomass accumulations within the fermentor **100** may be removed to maintain the overall biomass within the fermentor **100** within a defined range or above or below a defined threshold. In at least some instances, biomass removed from the fermentor **100** via the at

- **5** least one biomass removal fluid connection **208** may include one or more useful compounds. For example, the biological organisms within the excess biomass may contain an amount of one or more intracellular lipids or similar compounds useful in the production of a biofuel such as biodiesel.
- The one or more liquids 202 may include any liquid suitable for **10** sustaining or delivering one or more nutrients to the microbiological organisms within the fermentor **100.** Such liquids 202 may include, but are not limited to, solutions containing water, one or more alcohols, minerals, one or more nitrogen containing compounds, one or more phosphorus containing compounds, and the like. In at least some instances, one or more fluid movers
- **15 216** are used to deliver the one or more liquids 202 to the one or more liquid distributors **130** either in or fluidly coupled to the fermentor **100** in a controlled manner and at a pressure that is greater than atmospheric pressure. The one or more fluid movers **216** can include any type of pump or similar device capable of transferring a liquid between two points. Example fluid movers **216**
- 20 include, but are not limited to, centrifugal pumps, positive displacement pumps, progressing cavity pumps, double diaphragm pumps, and the like. Other illustrative fluid movers **216** include, but are not limited to eductors, ejectors, and similar devices. The transfer of liquid 202 to the fermentor **100** can be flow controlled, pressure controlled, or controlled using combinations of pressure,
- **25** temperature, flow, level, flowrate, superficial velocity, or compositional analysis process variable data gathered from one or more points within the fermentor **100** or from one or more points within the fermentation system 200. In at least some instances, the transfer of liquid 202 **by** the fluid mover **216** can be controlled based on the measured concentration of one or more components or
- **30** compounds (e.g., one or more nitrogen containing nutrients) within the fermentor **100,** for example, the flow of liquid **206** transferred **by** the fluid mover **216** may be increased in response to a measured decrease in nutrient concentration within the fermentor **100.**
- In at least some instances, the flowrate of the liquid transferred **by 35** the fluid mover **216** can be based in whole or in part on maintaining the velocity of the downward fluid flow **116** in the downward flow path **114** within a defined

range that is able to transport the gas bubbles present in the multi-phase mixture downwardly along the downward flow path **114.** The velocity of the downward flow **116** in the downward flow path **114** can be measured using one or more contact or non-contact flow sensors. Example flow sensors can

- **5** include, but are not limited to, magnetic flowmeters, Doppler flowmeters, mass flowmeters, and the like. In such instances, the downward flow **116** in the downward flow path 114 can have a velocity of from about **0.1** feet/second (f/s) to about **15** f/s; from about 0.2 feet/second (f/s) to about **10** f/s; or from about **0.5** feet/second (f/s) to about **5** f/s.
- **10** Although depicted on the inlet side of the fermentor **100,** the fluid mover **216** may be disposed at any location where providing a downward flow **116** in the downward flow path **114** and an upward flow 120 in the upward flow path **118** is possible. For example, as shown in Figure 2, the fluid movers **216** may be fluidly coupled to the inlet of the fermentor **100** to provide a forced
- **15** downward flow **116** through the downward flow path **114** and a forced upward flow 120 through the upward flow path **118.** In another example, all or a portion of the fluid movers **216** may be fluidly coupled between the downward flow path **114** and the upward flow path **118** to provide an induced downward flow **116** along the downward flow path **114** and a forced upward flow 120 along the
- 20 upward flow path **118.** In yet another example, all or a portion of the fluid movers **216** may be fluidly coupled to the multi-phase mixture discharge fluid connection **138** to induce a downward flow **116** through the downward flow path 114 and to induce an upward flow 120 through the upward flow path **118.**

The one or more gas substrates 204 can include any gas, gases, **25** or combination of gases suitable for sustaining or delivering one or more nutrients to the biological organisms within the fermentor **100.** Such gases can

- include, but are not limited to, one or more gases containing carbon compounds. Such gases can include, but are not limited to, one or more gases containing C₁ carbon compounds such as methane or carbon monoxide. The
- **30** one or more gas substrates 204 may also include one or more gases used in the metabolic processes of the biological organisms within the fermentor **100.** Such gases can include, but are not limited to, oxygen, oxygen containing compounds and hydrogen. The one or more gas substrates 204 may be transferred to the fermentor **100** as a pure gas or as a gas mixture (e.g., syngas
- **35 -** a mixture of carbon monoxide and hydrogen). The one or more gas substrates 204 may be transferred to the fermentor **100** individually (e.g.,
methane and an oxygen containing gas such as air may be transferred individually to minimize the likelihood of formation of an explosive gas mixture external to the fermentor **100).**

- The one or more gas substrates 204 may optionally be transferred **5** to the fermentor **100** using a gas mover **218.** Example gas movers **218** include, but are not limited to, rotary lobe compressors, centrifugal compressors, screw compressors, and the like. The delivery pressure of the one or more gas substrates 204 depends upon a variety of factors including the operating pressure of the fermentor **100** and the pressure drop associated with the gas
- **10** distributor **132** used to distribute the one or more gas substrates 204 within the downward flow path 114. Similarly, the delivery flowrate of the one or more gas substrates may be manually or automatically controlled to maintain the concentration or level of dissolved gas within the fermentor **100** within a defined range (e.g., dissolved oxygen above at least 4 ppm) based at least in part on
- **15** the needs of the biological organisms present in the fermentor **100.** In at least some instances, the one or more gas substrates 204 can be delivered to the fermentor **100** at a pressure of from about **5** psig to about **600** psig; from about **5** psig to about **600** psig; from about **25** psig to about 400 psig; or from about **50** psig to about **300** psig.
- 20 Any number of gases may be introduced through a common gas distribution header **132** or any number of individual gas distribution headers **132.** Such gas distribution headers may introduce all of the gas substrate 204 at a single point within the fermentor **100** or may introduce portions of the gas substrate 204 at various locations throughout the fermentor **100.** In at least
- **25** some instances, the gas substrate 204 can include, but is not limited to, methane, carbon monoxide, hydrogen, or oxygen. In at least some instances, the feed rate of the gas substrate 204 can be referenced to the feed rate of the liquid media 202. For example, methane may be introduced as a gas substrate 204 at a rate of from about **0.1** grams of methane/liter of liquid media **(g/i)** to
- **30** about **100 g/l;** from about **0.5 g/I** to about **50 g/l;** or from about **1 g/I** to about **25 g/l.** Carbon monoxide **("CO")** may be introduced as a gas substrate 204 at a rate of from about **0.1** grams of CO/liter of liquid media **(g/1)** to about **100 g/l;** from about **0.5 g/I** to about **50 g/l;** or from about **1 g/I** to about **25 g/l.** Oxygen may be introduced as a gas substrate 204 at a rate of from about **1** grams of
- **35** oxygen/liter of liquid media **(g/1)** to about **100 g/l;** from about 2 **g/I** to about **50 g/l;** or from about **5 g/I** to about **25 g/l.** Hydrogen may be introduced as a gas

substrate 204 at a rate of from about **0.01** grams of hydrogen/liter of liquid media **(g/i)** to about **50g/l;** from about **0.1 g/I** to about **25 g/l;** or from about **1 g/I** to about **10 g/l.**

- Within the fermentor **100** the microbiological organisms will **5** metabolize at least a portion of the carbon containing compounds present in the multi-phase mixture. At least a portion of this process may include the production of additional microbiological organisms that increase the overall quantity of biomass present in the fermentor **100.** Left uncontrolled, the biomass within the fermentor **100** can accumulate to a point such that one or
- **10** more operational aspects of the fermentor **100** (e.g., flowrate, pressure drop, production of desired products, etc) is compromised or adversely affected **by** the presence of the excess biomass. In such instances, the ability to remove at least a portion of the biomass present in the fermentor **100** is desirable. In at least some instances, at least a portion of the floor 124 of the fermentor **100**
- **15** may be sloped (e.g., a cone bottom) or shaped (e.g., an **ASME** dished head) such that biomass preferentially accumulates at a location within the fermentor **100** facilitating biosolids removal from the fermentor **100** via the at least one biomass removal fluid connection **208.** For example, an **ASME** dished head may facilitate the accumulation of biosolids at a central location of the head
- 20 proximate a fluid connection or similar device that permits the removal of the biosolids **208** from the fermentor **100** via the at least one biomass removal fluid connection **208.**

The backpressure subsystem **230** can include any number of devices, systems, or combinations thereof to maintain the pressure within the **25** fermentor **100** within a defined range. For example, the backpressure subsystem **230** may maintain the pressure in the fermentor **100** in a range that is at or above atmospheric pressure. In at least some instances, the backpressure subsystem **230** can maintain an upstream pressure (i.e., pressure in the fermentor **100)** of from about **1** psig to about **150** psig; about **1**

- **30** psig to about **100** psig; about **1** psig to about **75** psig; about **1** psig to about **50** psig; or about **1** psig to about **25** psig. Maintaining the fermentor **100** at a pressure greater than atmospheric pressure may advantageously improve the rate of mass transfer between the gas substrate bubbles, the liquid media, and consequently the microbiological organisms within the fermentor **100 by**
- **35** maintaining the partial pressure of gases used in the metabolic processes of the microbiological organisms at an elevated level in the fermentor **100** (i.e.,

gas substrate partial pressures greater than those achievable at atmospheric pressure).

At least a portion of the multi-phase mixture **136** that exits the fermentor **100** is received **by** the backpressure subsystem **230.** The flowrate of

- **5** the multi-phase mixture **136** exiting the fermentor **100** may be manually or automatically controlled. For example, the flowrate of the multi-phase mixture **136** exiting the fermentor **100** may be measured using a process element (not shown in Figure 2) such as a mass flowmeter, a magnetic flowmeter, an ultrasonic flowmeter, or similar. Such flow measurement can provide one or
- **10** more signals containing data representative of the multi-phase mixture discharge **136** flow, velocity, pressure, composition, or combinations thereof to a control subsystem **290.** The control subsystem **290** can include a local controller, a central control system, or distributed control system capable of providing a control output to a final control element such as a control valve (not
- **15** shown in Figure 2). The multi-phase mixture discharge **136** passes through the backpressure subsystem **230** and exits as a low pressure multi-phase mixture **232.** In at least some instances, the backpressure subsystem **230** can maintain an downstream pressure (i.e., pressure to the separation subsystem **250)** and the low pressure multi-phase mixture **232** can have a pressure of from about **1**
- 20 psig to about **150** psig; about **1** psig to about **100** psig; about **1** psig to about **75** psig; about **1** psig to about **50** psig; or about **1** psig to about **25** psig.

The backpressure subsystem **230** can include any number of pressure reducing devices including, but not limited to, orifices, pressure reducing valves, turbines, or any other device or system capable of providing a

- **25** known, controllable, pressure drop. In some instances, the pressure within the fermentor **100** may be manually or automatically varied, controlled, or adjusted based upon one or more control inputs provided to the backpressure subsystem **230.** In at least some instances, the backpressure subsystem **230** can include one or more turbines or similar devices that are capable of
- **30** providing a shaft output 234. Such a shaft output 234 may be used as an input to an energy recovery device **236,** for example a generator capable of providing electrical power for local consumption or provision to a local commercial, industrial, or residential electric distribution network.
- The separation subsystem **250** can include any number of **35** devices, systems, or combinations thereof to separate the low pressure multi phase mixture **232** into at least a gas effluent **252** and a liquid effluent 254. In

at least some instances, biosolids present in the low pressure multi-phase mixture **232** may be separated into a solids-containing effluent **256.** In at least some instances, at least a portion of the solids-containing effluent **256** from the separation subsystem **250** can be combined with the one or more liquids 202 to

5 provide a mixture **258** for return to the fermentor **100.** In at least some instances, the separation subsystem **250** can include one or more separators having an aggregate volume of at least about **10%** of the volume of the vessel **108;** at least about 20% of the volume of the vessel **108;** at least about 20% of the volume of the vessel **108;** or at least about 40% of the volume of the vessel

10 108.

The separation subsystem **250** can include one or more passive separators (e.g., one or more wet cyclones or the like) capable of separating the gas effluent **252** and the liquid effluent 254 from the low pressure multi phase mixture **232.** In at least some instances, the passive separator may also

15 include a solids separation section to separate at least a portion of the biosolids present in the low pressure multi-phase mixture **232.** In other instances, the separation subsystem **230** may include one or more active separation devices (e.g., a three phase rotary separator) capable of separating the gas effluent **252,** the liquid effluent 254, and the solids-containing effluent from the low 20 pressure multi-phase mixture **232.**

In at least some instances, the gas effluent **252** may include a mixture of one or more gas substrates (e.g., methane or carbon monoxide) and one or more gaseous byproducts (e.g., carbon dioxide) generated as a byproduct **by** the biological organisms in the fermentor **100.** In at least some

- **25** instances, the gas effluent **252** may be separated (not shown in Figure 2) and at least a portion of the one or more gas substrates recycled to the fermentor **100** for example as a gas substrate 204. In at least some instances, the gas effluent **252** may include one or more useful compounds. For example, the gas effluent **252** may contain an amount of one or more gaseous **C2.** hydrocarbon
- **30** compounds including, but not, limited to ethane, ethylene, propane, butane, and compounds based thereupon having value as either a finished product or as a raw material in a subsequent process. Such useful compounds may be separated from the gas effluent **252** prior to recycling at least a portion of the gas effluent **252** to the fermentor **100.**
- **35** In at least some instances, the liquid effluent 254 may include a mixture containing one or more liquids, nutrients, and the like introduced to the

fermentor with the liquid 202. In such instances, at least a portion of the liquid effluent 254 can be separated (not shown in Figure 2) and recycled to the fermentor **100** for example as a liquid media 202. In at least some instances, the liquid effluent 254 may include one or more useful compounds. For

- **5** example, the liquid effluent 254 may contain an amount of one or more liquid **C2.** hydrocarbon compounds including, but not, limited to alcohols, ketones, glycols, and other compounds based thereupon having value as either a finished product or as a raw material in a subsequent process. Such useful hydrocarbon compounds may be separated from the liquid effluent 254 prior to
- **10** disposing of the liquid effluent 254 or recycling at least a portion of the liquid effluent 254 back to the fermentor **100.**

In at least some instances, the solids-containing effluent **256** may include a slurry or mixture containing one or more liquids and biomass removed from the fermentor **100.** In such instances, it may be desirable to recycle at

- **15** least a portion of the solids-containing effluent **256** back to the fermentor **100.** The recycle of biomass to the fermentor **100** may advantageously serve to reseed the microbiological colony within the fermentor **100.** Although not shown in Figure 2, at least a portion of the solids-containing effluent **256** can be recycled directly to the downward flow path **114** in the fermentor **100.** At least a
- 20 portion of the solids-containing effluent **256** can be mixed with the one or more liquids 202 and recycled to the fermentor **100.** In at least some instances, the solids-containing effluent **256** may include one or more useful compounds. For example, the biological organisms within the solids-containing effluent **256** may contain an amount of one or more intracellular lipids or similar compounds
- **25** useful in the production of a biofuel such as biodiesel. Although not shown in Figure 2, in such instances, at least a portion of the solids-containing effluent **256** may be removed from the separation subsystem **230** for subsequent processing into one or more desired products.
- The thermal subsystem **270** can include any number of devices, **30** systems, or combinations thereof to add thermal energy to or to remove thermal energy from the multiphase fluid in the fermentor **100.** In at least some instances, the fermentation that occurs within the fermentor **100** generates heat as a byproduct. Left uncontrolled, such heat can adversely affect the metabolism or health of the microbiological organisms within the fermentor **100.**
- **35** Alternatively, microbiological organisms may also have a temperature below which the metabolism or health of the organism is adversely affected. As such,

the biological organisms within the fermentor **100** have a defined temperature range providing optimal growth and metabolic conditions. In at least some instances, the multi-phase mixture within the fermentor **100** can be maintained at a temperature of about 130°F or less; about 120°F or less; about 110°F or

- **5** less; about 100°F or less; about 95°F or less; about 90°F or less; about 85°F or less; or about 80°F or less using the thermal subsystem 270. In at least some instances, the multi-phase mixture within the fermentor **100** can be maintained at a temperature of from about 55°F to about 120°F; about 60°F to about **¹¹**00F; about **11** 00F to about 1200 F; about **1** 00⁰ F to about 120⁰ F; about **65⁰ F** to
- **10** about **100[°]F**; about 65[°]F to about 95[°]F; or about 70[°]F to about 90[°]F using the thermal subsystem **270.**

In at least some instances, one or more thermal transfer surfaces **128** may be disposed at least partially within the downward flow path **114** to alter, adjust, or control the temperature of the downward flow **116** of the multi

- **15** phase mixture therein. Similarly, one or more thermal transfer surfaces 140 may be disposed at least partially within the upward flow path **118** to alter, adjust, or control the temperature of the upward flow 120 of multi-phase mixture therein.
- In at least some instances, such thermal transfer surfaces **128,** 20 140 may include one or more piezoelectric surfaces which provide cooling upon passage of an electric current therethrough. Such thermal transfer surfaces **128,** 140 may include one or more resistive surfaces (e.g., Calrod heaters) which provide heating to increase the temperature of the multi-phase mixture upon passage of an electric current therethrough. In such instances, the
- **25** thermal subsystem **270** can include one or more systems to control the flow of electrical power to the thermal transfer surfaces **128,** 140 based at least in part on the measured temperature of the multi-phase mixture in the fermentor **100.**

In other instances, such thermal transfer surfaces **128,** 140 can include a number of reservoirs or conduits positioned within the downward flow **30** path 114, the upward flow path 120 or both, and through which thermal transfer media at either a reduced temperature or an elevated temperature is circulated to variously cool or heat the multi-phase mixture in the fermentor **100.** The thermal transfer media can include any fluid, liquid, or gas capable of transporting or otherwise conveying thermal energy to or thermal energy from

35 the multi-phase mixture in the fermentor **100.** In such instances, the thermal subsystem **270** can include one or more systems or devices to remove thermal

energy from the thermal transfer media (e.g., for cooling the multi-phase mixture in the fermentor **100)** or impart thermal energy to the thermal transfer media (e.g., for warming the multi-phase mixture in the fermentor **100).**

- In at least one instances, the thermal transfer media can include **5** water or an aqueous solution of water and one or more corrosion, scale, and microbiological inhibitors. In such instances, the thermal subsystem **270** can include one or more systems for removing thermal energy from the thermal transfer media to cool the multi-phase mixture. For example, an open-loop evaporative cooling tower or a closed loop air cooler may be used to cool the
- **10** thermal transfer media. In such instances, the thermal subsystem **270** may optionally include one or more systems for imparting thermal energy to the thermal transfer media to warm the multi-phase mixture. For example, a natural gas or electric water heater or furnace may be used to warm the thermal transfer media.
- **15** The thermal transfer media can be provided **by** the thermal subsystem **270** via one or more thermal media distribution networks **272.** The one or more thermal media distribution networks **272** can include an electrical distribution network where electric heating and cooling thermal transfer surfaces **128,** 140 are provided. The one or more thermal media distribution
- 20 networks **272** can include a network of fluid conduits where a liquid thermal transfer media (e.g., water) is provided to the thermal transfer surfaces **128,** 140. Branches 210, 214 from the thermal media distribution network **272** can be used to supply thermal transfer media to the thermal transfer surfaces **128,** 140. For example, a branch **210** can be used to supply thermal transfer media
- **25** from the thermal media distribution network **272** to the thermal transfer surface **128** disposed in the downward flow path 114, and a branch 214 can be used to supply thermal transfer media from the thermal media distribution network **272** to the thermal transfer surface 140 disposed in the upward flow path 120.
- The thermal transfer media can be collected and at least partially **30** returned to the thermal subsystem **270** via one or more thermal media collection networks 274. The one or more thermal media collection networks 274 can include a collection of networked fluid conduits 212, **216** where a liquid thermal transfer media (e.g., water) is removed from the thermal transfer surfaces **128,** 140. Branches from the thermal media collection network 274
- **35** can be used to collect thermal transfer media from the thermal transfer surfaces **128,** 140. For example, a branch 212 can be used to return thermal transfer

media from the thermal transfer surface **128** disposed in the downward flow path 114 to the thermal media collection network 274, and a branch **216** can be used to return thermal transfer media from the thermal transfer surface 140 disposed in the upward flow path 120 to the thermal media collection network **5** 274.

In at least some instances, all or a portion of the fermentation process may be at least partially automatically controlled using a control subsystem **290.** The control subsystem **290** may collect process related information provided **by** one or more process elements in the form of signals

- **10** containing analog or digital data representing one or more process variables. For instance, the control subsystem can collect process related signals using one or more process elements including, but not limited to, mass flow sensors, volumetric flow sensors, temperature sensors, pressure sensors, level sensors, analytical sensors (e.g., dissolved oxygen sensors, biological oxygen demand
- **15** or "BOD" sensors, **pH** sensors, conductivity sensors, and the like) or any other device capable of providing a signal containing data representative of one or more process related condition within the fermentor **100.**

The control subsystem **290** may execute one or more sets of instructions controlling, altering, or adjusting one or more aspects of the

- 20 fermentation process based at least in part on the process variable signals received from the process elements. Such instructions may result in the generation of one or more control output signals **by** the control subsystem **290.** The control output signals can be transmitted from the control subsystem **290** to one or more final control elements such as block valves, control valves, motors,
- **25** variable speed drives, etc. The interaction between the final control elements and the fermentation process can, in turn, provide the control subsystem **290** with a high degree of relatively accurate control of the fermentation process.

For example, responsive to the receipt of one or more signals containing data indicative of the temperature of the multi-phase mixture in the

- **30** fermentor **100,** the control subsystem **290** may initiate, alter, or cease the flow of thermal transfer media to one or more thermal transfer surfaces **128,** 140. Similarly, responsive to the receipt of one or more signals containing data indicative of the dissolved oxygen level of the multi-phase mixture in the fermentor **100,** the control subsystem **290** may increase, decrease, or maintain
- **35** the flow of the oxygen containing gas substrate 204 to the fermentor **100.** Although only two illustrative examples are provided herein, any flow, level,

pressure, analytical value, or the like that is appropriate to the fermentation process may be similarly controlled **by** the control subsystem **290** using one or more appropriate process sensors and one or more appropriate final control elements.

- **5** Figure **3** shows a cross-sectional elevation view of an example fermentor **300.** The fermentor **300** includes one or more internal structures including one or more structures 302a-302c (only three shown, any number possible, collectively "structures **302")** disposed at least partially within the downward flow path 114 and one or more structures 304a-304d (only four
- **10** shown, any number possible, collectively "structures 304") disposed at least partially within the upward flow path 120. Such structures can advantageously promote in both the downward and upward flow paths **114, 118** mass transfer between the gas bubbles and liquid in the multi-phase mixture and between the multi-phase mixture and the biological organisms. Such structures can
- **15** advantageously promote uniformity of gas bubbles within the multi-phase mixture **by** facilitating a more even distribution within the downward and upward flow paths 114, **118,** and promote additional contact time between the gas substrate bubbles present in the multi-phase mixture and the biological organisms within the fermenter **300.** The fermentor **300** has a conical bottom
- 20 124 that advantageously promotes the collection of excess biomass **306** at a location in the fermentor **300** proximate the at least one biomass removal fluid connection **208.**

The fermentor **300** includes one hollow fluid conduit 102 disposed within a vessel **108.** The at least one fluid mover **216** is disposed on the inlet **25** side of the downward flow path **114,** with liquid flow distributed throughout the downward flow paths 114 in the fermentor **300** via a plurality of fluid connections 130a-130n (collectively, "fluid connections **130")** disposed about the top **126** of the fermentor **300.** The fluid connections **130** are directly, fluidly, coupled to at least a portion of the downward flow paths 114. The at least one **30** fluid mover **216** provides a generally downward flow **116** of liquid **308** within the

downward flow path **114.**

The fermentor **300** includes a number of gas distributors 132a 132n (collectively, "gas distributors **132")** disposed about the fermentor **300.** One or more substrate gases 204 are introduced to the downward flow path

35 114 in the form of gas bubbles **310.** In at least some instances, the gas bubbles **310** can include one or more gas substrates. Within the downward

flow path **114,** the liquid **308** and the gas bubbles **310** mix and otherwise combine to form the multi-phase mixture **312.** Within the downward flow path 114, the gas substrate and nutrients in the multi-phase mixture are used **by** the biological organisms to form a biomass 314 on most if not all of the internal **5** surfaces forming the downward flow path **114.**

In at least some instances, the liquid **308** introduced to the downward flow path 114 can include an aqueous solution containing one or more nutrients, trace elements, minerals, and the like that are capable of supporting the growth and development of a biomass at least within the

- **10** downward flow path 114. In at least some instances, the liquid **308** can include dissolved nitrogen or one or more nitrogen containing compounds capable of providing a dissolved nitrogen concentration of from about **1** milligram/liter (mg/I) to about **30** mg/I; from about **1** milligram/liter (mg/I) to about 20 mg/I; or from about **1** milligram/liter (mg/I) to about **10** mg/I. In at least some instances,
- **15** the liquid **308** can include dissolved phosphorous or one or more phosphorous containing compounds capable of providing a dissolved phosphorous concentration of from about **1** mg/I to about **30** mg/I; from about **1** milligram/liter (mg/I) to about 20 mg/I; or from about **1** milligram/liter (mg/I) to about **10** mg/I. In at least some instances, the gas introduced to the downward
- 20 flow path 114 can include any number of substrate gases including, but not limited to, at least one of methane, carbon monoxide, oxygen, oxygen containing compounds, hydrogen and hydrogen containing compounds. In at least some instances, the gas(es) may be supplied at conditions (e.g., concentration, temperature and pressure) that advantageously support the
- **25** dissolution of the gases in the liquid **308** to form the multi-phase mixture **312.** In at least some instances, the addition of the substrate gases may be manually or automatically controlled to maintain any defined level of dissolved gas in the multi-phase mixture within the fermentor **300.** In some instances, although not shown in Figure **3,** the gas bubbles **310** may be introduced within the downward
- **30** flow path **114,** the upward flow path 120, or both. The gas bubbles **310** introduced at different points in the fermentor **300** may or may not have the same composition or be at the same temperature or pressure. In at least some instances, the composition of the gas bubbles **310** may be adjusted based at least in part on the location within the fermentor **300** at which the gas bubbles
- **35 310** are introduced. For example, the substrate gas may have differing concentrations in gas bubbles **310** introduced via one or more gas distributors

132 at various points in the fermentor **300.** In at least some instances, the temperature of the gas bubbles **310** may be adjusted based at least in part on the location within the fermentor **300** at which the gas bubbles **310** are introduced. In at least some instances, the pressure of the gas bubbles **310**

5 may be adjusted based at least in part on the location within the fermentor **300** at which the gas bubbles **310** are introduced.

In at least some instances, at least one of the composition, temperature, or pressure of the gas bubbles **310** may be adjusted or otherwise controlled to maintain a dissolved methane concentration within the multi-phase

- **10** mixture of from about **0.1mg/I** to about **100** mg/I; from about **0.5** mg/I to about **50** mg/I; from about **1** mg/I to about 20 mg/I; or from about **1** mg/I to about **10** mg/I. In at least some instances, at least one of the composition, temperature, or pressure of the gas bubbles **310** may be adjusted or otherwise controlled to maintain a dissolved carbon monoxide concentration within the multi-phase
- **15** mixture of from about **0.1** mg/I to about **100** mg/I; from about **0.5** mg/I to about **50** mg/I; from about **1** mg/I to about 20 mg/I; or from about **1** mg/I to about **10** mg/I. In at least some instances, at least one of the composition, temperature, or pressure of the gas bubbles **310** may be adjusted or otherwise controlled to maintain a dissolved oxygen concentration within the multi-phase mixture of
- 20 from about **0.1** mg/I to about **100** mg/I; from about **0.5** mg/I to about **50** mg/I; from about **1** mg/I to about 20 mg/I; or from about **1** mg/I to about **10** mg/I. In at least some instances, at least one of the composition, temperature, or pressure of the gas bubbles **310** may be adjusted or otherwise controlled to maintain a dissolved hydrogen concentration within the multi-phase mixture of from about
- **25 0.1** mg/I to about **50** mg/I; from about **0.5** mg/I to about **25** mg/I; from about **1** mg/I to about **10** mg/I; or from about **1** mg/I to about **5** mg/I.

In at least some instances, the structures **302** may be disposed at least partially within the downward flow path 114. Although the gas bubbles **310** would ordinarily tend to flow upward within the downward flow path **114,**

- **30** the fluid velocity within the downward flow path tends to "push" the substrate gas bubbles **310** downward at a velocity that is less than the fluid velocity. The extended residence time of the gas bubbles **310** in the downward flow path **¹¹⁴** advantageously provides an extended opportunity for the gas(es) present in the gas bubbles **310** to dissolve into the multi-phase mixture and consequently for
- **35** the biological organisms existent in the biomass 314 in the downward flow path 114 to absorb the dissolved substrate gas(es) from the multi-phase mixture.

The structures **302** may advantageously enhance this effect as gas bubbles **310** trapped beneath and around the structures will also experience the previously elaborated attendant benefits from an extended residence time within the downward flow path 114. The extended residence time is particularly

- **5** advantageous in supporting the development of larger quantities of biomass 314 within the downward flow path. The larger quantities of biomass 314 **so** developed may be at least partially attributable to the enhanced levels of dissolved substrate gas(es) present in the downward flow path 114.
- The structures **302** can include any number, type, size, or **10** configuration of projecting member on or recessed detent in fluid contact with the downward flow path **114. All** or a portion of the structures **302** can be similar or different dependent upon the function of the structure **302.** For example, a number structures 302a sharing a common first design type that promotes mixing of the multi-phase mixture and increases the residence time of
- **15** the gas bubbles **310** may be disposed within the downward flow path 114. **A** number of structures **302b** sharing a common second design type providing extended surfaces that promote the growth or formation of biomass 314 may be disposed within the downward flow path 114.
- In at least some instances, the structures 304 may be disposed at 20 least partially within the upward flow path 120. Although the gas bubbles **310** would flow upward within the upward flow path 114, additional upward velocity is imparted to the gas bubbles **310 by** the velocity of the upward fluid flow 120 within the upward flow path **118** which tends to "push" the gas bubbles **310** upward at a velocity that exceeds the normal rise rate of the gas bubbles **310.**
- **25** The structures 304 can advantageously hinder or otherwise impede the progress of the gas bubbles **310** in the upward flow path **118,** thereby increasing the residence time of the gas bubbles **310** within the upward flow path **118.** The extended residence time of the gas bubbles **310** in the upward flow path **118** advantageously provides an extended opportunity for the gas(es)
- **30** present in the gas bubbles **310** to dissolve into the multi-phase mixture and consequently for the biological organisms existent in the biomass 314 in the upward flow path **118** to absorb the dissolved substrate gas(es) from the multi phase mixture. The structures 304 may advantageously enhance this effect as gas bubbles **310** trapped beneath and around the structures 304 will also
- **35** experience the previously elaborated attendant benefits from an extended residence time within the upward flow path **118.** The extended residence time

is particularly advantageous in supporting the development of larger quantities of biomass 314 within the upward flow path **118.** The larger quantities of biomass 314 so developed may be at least partially attributable to the enhanced levels of dissolved substrate gas(es) present in the upward flow path **5 118.**

The structures 304 can include any number, type, size, or configuration of projecting member on or recessed detent in fluid contact with the upward flow path **118. All** or a portion of the structures 304 can be similar or different dependent upon the function of the structure. For example, a

- **10** number structures 304a sharing a common first design type that promotes mixing of the multi-phase mixture and increases the residence time of the gas bubbles **310** may be disposed within the upward flow path **118. A** number of structures 304b sharing a common second design type providing extended surfaces that promote the growth or formation of biomass 314 may be disposed
- **15** within the upward flow path 114.

As biomass 314 accumulates within the downward flow path **114** and the upward flow path **118,** excess biomass will slough or otherwise detach from the interior surfaces of the fermentor **300.** At least a portion of the excess biomass will fall to the bottom on the fermentor **300** where the biomass will

- 20 accumulate. At least a portion of the excess biomass will be carried or transported from the fermentor **300** with the multi-phase mixture **136.** Excess biomass accumulations within the bottom of the fermentor **300** may be removed as biosolids. At least a portion of such biosolids may be further processed to yield one or more fungible products such as biofuels or **C2.** hydrocarbons useful
- **25** as either finished products or as raw materials for subsequent processing. At least a portion of the excess biomass removed from the fermentor **300** may be used to "re-seed" or propagate the microbiological organisms present in the biosolids in other fermentors **300.**
- At least a portion of the excess biomass carried from the **30** fermentor **300** with the multi-phase mixture **136** may be separated, for example in the separation subsystem **250,** and at least a portion recycled to the fermentor **300** at least to assist in establishing the microbiological colony within the fermentor **300.**
- In at least some instances, the microbiological organisms used to **35** ferment gaseous carbon-containing feedstocks employ a culture primarily comprising a **C1** metabolizing non-photosynthetic microorganism. Such

fermentation systems may use one or more species of C_1 metabolizing microorganisms that are prokaryotes or bacteria, such as Methylomonas, Methylobacter, Methylococcus, Methylosinus, Methylocystis, Methylomicrobium, Methanomonas, Methylophilus, Methylobacillus,

- **5** Methylobacterium, Hyphomicrobium, Xanthobacter, Bacillus, Paracoccus, Nocardia, Arthrobacter, Rhodopseudomonas, or Pseudomonas. In some instances, the C₁ metabolizing bacteria may include a methanotroph or a methylotroph. Preferred methanotrophs include Methylomonas, Methylobacter, Methylococcus, Methylosinus, Methylocystis, Methylomicrobium,
- **10** Methanomonas, or a combination thereof. Exemplary methanotrophs include Methylomonas sp. 16a **(ATCC** PTA 2402), Methylosinus trichosporium (NRRL B-II,1 **96),** Methylosinus sporium (NRRL B-lI, **197),** Methylocystis parvus (NRRL B-lI, **198),** Methylomonas methanica (NRRL B-5 **11,199),** Methylomonas alb us (NRRL B-Il ,200), Methylobacter capsulatus (NRRL B-1 1,201),
- **15** Methylobacterium organophilum **(ATCC 27,886),** Methylomonas sp. **AJ-3670** (FE RM P-2400), Methylomicrobium alcaliphilum, Methylocella silvestris, Methylacidiphilum infernorum, Methylibium petroleiphilum, Methylosinus trichosporium **OB3b,** Methylococcus capsulatus Bath, Methylomonas sp. 16a, Methylomicrobium alcaliphilum 20Z, or a high growth variants thereof.
- 20 Preferred methylotrophs include Methylobacterium extorquens, Methylobacterium radiotolerans, Methylobacterium populi, Methylobacterium chloromethanicum, Methylobacterium nodulans, or a combination thereof.

Microorganisms capable of metabolizing **C1** compounds found in syngas include, but are not limited to Clostridium, Moorella, Pyrococcus,

- **25** Eubacterium, Desulfobacterium, Carboxydothermus, Acetogenium, Acetobacterium, Acetoanaerobium, Butyribaceterium, Peptostreptococcus, or combinations thereof may also be used. Exemplary methylotrophs include Clostridium autoethanogenum, Clostridium Ijungdahli, Clostridium ragsdalei, Clostridium carboxydivorans, Butyribacterium methylotrophicum, Clostridium
- **30** woodii, Clostridium neopropanologen, or a combination thereof. In some instances, C₁ metabolizing microorganisms are eukaryotes such as yeast, including Candida, Yarrowia, Hansenula, Pichia, Torulopsis, or Rhodotorula. In other instances, the **Ci** metabolizing non-photosynthetic microorganism is an obligate C₁ metabolizing non-photosynthetic
- **35** microorganism, such as an obligate methanotroph or methylotroph. In some instances, the C₁ metabolizing non-photosynthetic microorganism is a

recombinant microorganism comprising a heterologous polynucleotide encoding a fatty acid producing enzyme, a formaldehyde assimilation enzyme, or a combination thereof.

- Figure 4 shows a cross-sectional elevation view of an example **5** fermentor 400. The bottom 124 of the fermentor 400 includes a plurality of conical sections, each of which can accumulate excess biomass **306.** In such instances, at least one biomass removal fluid connection **208** can be disposed proximate to the location on the bottom 124 at which the excess biomass accumulates to facilitate the removal of the excess biomass **306.** The
- **10** fermentor 400 includes six or more hollow fluid conduits 102a-1 **02f** (collectively, "hollow fluid conduits 102") disposed within the vessel **108.** At least one fluid mover **216** is disposed on the inlet side of the downward flow path **114,** with liquid flow distributed throughout the downward flow paths **114** via a plurality of fluid connections 130a-130f (collectively, "fluid connections **130")** disposed
- **15** about the fermentor top **126.** Each of the fluid connections **130** is directly, fluidly, coupled to the downward flow path 114. The at least one fluid mover **216** provides a generally downward flow of liquid **308** within the downward flow path **114.**
- The multi-phase mixture **136** exits the fermentor 400 via a plurality 20 of multi-phase mixture discharge fluid connections **138,** each of the multi-phase mixture discharge fluid connections directly fluidly coupled to the upward flow paths **118.** Each of the multi-phase mixture discharge fluid connections **138** is fluidly coupled to a multi-phase mixture discharge fluid conduit 402 that is used to convey the multi-phase mixture from the upward flow paths **118** to the
- **25** backpressure subsystem **230.**

The backpressure subsystem **230** shown in Figure 4 includes at least one multi-phase turbine 404. The pressure of the relatively higher pressure multi-phase mixture removed from the upward flow paths **118** is reduced through the multi-phase turbine 404 to provide a relatively lower

- **30** pressure multi-phase mixture that is directed away from the backpressure system **230** via one or more fluid conduits 406. At least a portion of the relatively lower pressure multi-phase mixture can be subsequently introduced to the separation subsystem **250.** In at least some instances, the multi-phase turbine 404 may be physically coupled to one or more energy recovery devices
- **35** (not shown in Figure 4) such as an electrical generator to recover at least a portion of the energy released **by** reducing the pressure of the multi-phase

mixture from the relatively higher pressure to the relatively lower pressure. In at least some instances, the physical coupling of the multi-phase turbine 404 and the energy recovery device may be accomplished using a shaft 408 or similar connection capable of transferring mechanical power. The backpressure

5 subsystem **230** can reduce or lower the pressure of the multi-phase mixture exiting the upward flow paths **118 by** about 120 psig or less; about **90** psig or less; about **60** psig or less; about **30** psig or less; or about **15** psig or less. The relatively lower pressure multi-phase mixture exiting the

backpressure subsystem **230** via the one or more fluid conduits 406 can in **10** some instances be introduced to the separation subsystem **250.** Within the separation subsystem **250,** the relatively lower pressure multi-phase mixture can be separated into a plurality of phases. In some instances, the relatively lower pressure multi-phase mixture may be separated at least into a gas phase **252** containing one or more desired products (e.g., **C2.** hydrocarbons such as

15 ethane and ethylene), one or more unconsumed gas substrates (e.g., methane or carbon monoxide), or one or more metabolic or chemical byproducts (e.g., carbon dioxide). In some instances, the relatively lower pressure multi-phase mixture may be separated at least into a liquid phase 254 containing one or more desired products (e.g., **C2.** hydrocarbons such as alcohols and ketones)

20 one or more unconsumed liquid phase nutrients, or one or more metabolic or chemical byproducts. In some instances, the relatively lower pressure multi phase mixture may be separated at least into a solids containing phase **256** containing one or more desired biosolids products (e.g., intracellular lipids useful as a biofuel) or one or more metabolic or chemical byproducts (e.g.,

25 excess biomass).

Figure **5** shows a cross-sectional elevation view of an example fermentor system **500.** The fermentor **500** includes a plurality of hollow fluid conduits 102 that extend completely through (i.e., from the top **126** to the bottom 124) of the vessel **108.** The upward flow paths **118** are fluidly coupled

- **30** to the downward flow paths **114** via one or more fluid conduits **502.** In at least some instances, one or more isolation devices 504 may be disposed in at least a portion of the one or more fluid conduits **502.** In at least some instances, the one or more fluid conduits **502** are fluidly coupled to at least a portion of the downward flow paths 114 using one or more fluid connections **506.** In at least
- **35** some instances, the one or more fluid conduits **502** are fluidly coupled to at least a portion of the upward flow paths **118** using one or more fluid

connections **508.** The one or more isolation devices 504 and the one or more fluid connections **506, 508** advantageously permit the isolation of one or more hollow fluid conduits 102. The ability to selectively isolate the hollow fluid conduits 102 advantageously permits routine maintenance and cleaning of the

5 hollow fluid conduits 102 without requiring the entire fermentor **500** be taken out of service.

Two different systems are shown coupling the downward flow path **114** and the upward flow path **118.** In a first instance, fluid conduits **502** are shown directly fluidly coupling the downward flow path 114 and the upward

- **10** flow path **118.** Such a direct fluid coupling installation may be used where the forced fluid flow or induced fluid flow is used in both the downward flow path **114** and the upward flow path **118.** For example, a direct fluid coupling system may be advantageous where the one or more fluid movers **216** are fluidly coupled to the inlet fluid connection **130** of the downward flow path **114** or are
- **15** fluidly coupled to the outlet fluid connection **138** of the upward flow path **118.** In this instance, excess biomass **306** can accumulate within the fluid conduits **502.** Such accumulated excess biomass **306** may be removed using the at least one biomass removal fluid connection **208.**
- In a second instance, one or more fluid movers **216** are fluidly 20 coupled between the downward flow path **114** and the upward flow path **118.** Such a fluid coupling provides an induced downward flow **116** in the downward flow path **114** and a forced upward flow 120 in the upward flow path **118.** In at least some instances, the one or more fluid movers **216** may be fluidly coupled to the downward flow path 114 using one or more fluid connections (e.g.,
- **25** flanged, threaded, quick connect, etc.) **506.** In at least some instances, the one or more fluid movers **216** may be fluidly coupled to the upward flow path using one or more fluid connections (e.g., flanged, threaded, quick connect, etc.) **508.** In at least some instances, excess biomass **306** drawn into the

one or more fluid movers **216** may be trapped or otherwise accumulated in one

- **30** or more accumulators **510.** Example accumulators **510** include, but are not limited to, one or more single or multi-stage filtration devices such as cartridge filters, self-cleaning filters, bag filters, basket filters, or combinations thereof. Example accumulators **510** can include, but are not limited to, one or more hydrocyclones or the like. In at least some instances, at least a portion of the
- **35** excess biomass **306** can be removed from the accumulator **510** using the at least one biomass removal fluid connection **208.** Although not shown in Figure

5, in at least some instances, one or more biomass removal fluid connections **208** may be disposed on the vessel **108.** For example, one or biomass removal fluid connections **208** may be disposed on the bottom 124 of the vessel **108** to removed accumulated biosolids **306.**

- **5** The fermenter **500** is also equipped with a plurality of gas distribution headers **132. A** first gas distribution header 132a is disposed in the downward flow path 114. **A** second gas distribution header **132b** is disposed in the upward flow path **118.** Such an arrangement advantageously provides the ability to replenish or increase a gas substrate depleted from the multi-phase
- **10** mixture during the passage through the downward flow path **114** prior to the multi-phase mixture entering the upward flow path **118.** For example, if methane is being used as a gas substrate, and the methane concentration in the multi-phase mixture exiting the downward flow path is below a defined value, additional methane may be added to the multi-phase mixture in the
- **15** upward flow path **118** using the gas distribution header **132b.** The second gas distribution header **132b** may advantageously provide the ability to raise different types of microorganisms at different locations within the fermentor **500.** For example, introducing a first gas substrate via the first gas distribution header 132a may promote the growth of first gas substrate metabolizing
- 20 microorganisms in the downward flow path 114. Introducing a second gas substrate via the second gas distribution header **132b** may promote the growth of second gas substrate metabolizing microorganisms in the upward flow path 114. The ability to feed a plurality of gas substrates in the same fermentor **500** may advantageously expand the product matrix achievable in the fermentor
- **25 500.**

Figure **6** shows a cross-sectional elevation view of an example fermentor system **600.** The bottom of the vessel **108** includes a plurality of dished heads (e.g., **ASME** dished heads, each of which can accumulate excess biomass **306.** In such instances, at least one biomass removal fluid connection

- **30 208** can be disposed proximate the location on the bottom 124 at which the excess biomass accumulates to facilitate the removal of the excess biomass **306.** The fermentor **602** includes a plurality of hollow fluid conduits 102a-1 **02b** (collectively, "hollow fluid conduits 102") disposed within the vessel **108.**
- At least one fluid mover **216** is fluidly coupled to the upward flow **35** path **118** via one or more multi-phase mixture discharge fluid connections **138.** Such an arrangement, where both the downward flow path 114 and the upward

flow path **118** are fluidly coupled to the suction side of the at least one fluid mover **216** can at least partially induce a fluid flow through both the downward flow path **114** and the upward flow path **118.** Although not shown in Figure **6,** in at least some instances, one or more optional additional fluid movers **216** may

5 be fluidly coupled to the downward flow path 114 via one or more liquid distribution headers 130a-1 30c. The at least one fluid mover **216** is fluidly coupled to the

A plurality of gas distribution headers 132a-1 **32b** (collectively "gas distribution headers **132")** is in fluid communication with the downward flow **10** path **114** of the fermentor **602.** Such an arrangement advantageously provides the ability to replenish or increase a gas substrate depleted from the multi phase mixture during the passage through the downward flow path **114** prior to the multi-phase mixture entering the upward flow path **118.** For example, if methane is being used as a gas substrate, and the methane concentration in

- **15** the multi-phase mixture exiting the downward flow path is below a defined value, additional methane may be added to the multi-phase mixture in the upward flow path **118** using the gas distribution header **132b.** The second gas distribution header **132b** may advantageously provide the ability to raise different types of microorganisms at different locations within the fermentor **602.**
- 20 For example, introducing a first gas substrate via the first gas distribution header 132a may promote the growth of first gas substrate metabolizing microorganisms in the downward flow path 114. Introducing a second gas substrate via the second gas distribution header **132b** may promote the growth of second gas substrate metabolizing microorganisms in the upward flow path
- **25** 114. The ability to feed a plurality of gas substrates in the same fermentor **602** may advantageously expand the product matrix achievable in the fermentor **602.**

Figure **7** shows a high level method of operation of a fermentation system using one or more fermentors **100** in one or more fermentor systems **30 300,** 400, **500, 600** described in detail above with regard to Figures **1-6.** Such systems advantageously introduce one or more liquid media containing one or more nutrients and one or more gaseous substrates in a downward flow path 114 to provide a multi-phase mixture having an overall downward flow **116** therein. The fluid velocity of the multi-phase mixture within the downward

35 flowpath **114** is sufficient to cause the gas substrate bubbles present in the multi-phase mixture to flow in a downward direction. However, the tendency for

the gaseous substrate bubbles to rise within the multi-phase mixture advantageously extends the residence time of the gaseous substrate bubbles within the downward flow path 114, thereby enhancing mass transfer and subsequent microbiological uptake of the gaseous substrate in the downward

- **5** flow path. After passing through the downward flow path **114,** the multi-phase mixture enters the upward flow **118** path where additional mass transfer and microbiological uptake may occur. The multi-phase mixture is removed from the fermentor and optionally pressure reduced and separated to provide one or more desired materials. The method commences at **702.**
- **10** At 704 the gaseous substrate is dispersed within the liquid media to form the multi-phase mixture. Such dispersion may occur at or near the inlet or beginning of the downward flow path 114, although additional quantities of gaseous substrate may be dispersed at other locations in the downward flow path **114,** the upward flow path **118,** or both. In some instances, gaseous
- **15** substrate may be dispersed at multiple points within the downward flow path **114,** upward flow path **118,** or both and the gaseous substrate at each dispersion point may have the same or a different temperature, pressure, composition, or combinations thereof. The ability to vary physical or compositional properties of the gaseous substrate at different locations within
- 20 the fermentor advantageously permits the tailoring of the gaseous substrate not only to the specific microbiological species present in the fermentor, but also to the specific location of the microbiological species within the fermentor based on the dispersion point of the gaseous substrate within the fermentor.

At **706** the multi-phase mixture provides a downward flow **116 25** within the downward flow path 114. The substrate gas bubbles will tend to rise within the downward flow path at a gas bubble rise rate of "X" feet per second. The multi-phase mixture will have a superficial fluid velocity within the downward flow path 114 of "Y" feet per second. **By** maintaining the superficial fluid velocity of the multi-phase mixture above the gas bubble rise rate (i.e., Y **>**

- **30** X), the substrate gas bubbles will flow downward rather than upward in the downward flow path 114. **By** adjusting or otherwise controlling the flow rate of the multi-phase mixture in the downward flow path **114** to a velocity that only slightly exceeds the gas substrate bubble rise rate, the residence time of the gas bubbles in the downward flow path 114 can be increased. Such an
- **35** increase in residence time in the downward flow path **114** can advantageously improve mass transfer and microbiological uptake of the gas substrate 204 **by**

the microbiological organisms present in the downward flow path 114. In some instances, the velocity of the multi-phase mixture in the downward flow path 114 can be measured and controlled. For example the control subsystem **290** can alter, adjust or control the fluid velocity of the multi-phase mixture in the

- **5** downward flow path 114 to a defined range slightly in excess of the rise rate of the substrate gas bubbles in the downward flow path 114. In some instances, the temperature, pressure, or composition of the gas substrate 204 may be altered, adjusted or controlled via the control subsystem **290** to maintain a desired gas substrate bubble size within the downward flow path 114. In other
- **10** instances, the temperature, pressure, or composition of the gas substrate 204 may be altered, adjusted or controlled via the control subsystem **290** to maintain the concentration of one or more gas substrate components (e.g., methane, carbon dioxide, hydrogen, oxygen, nitrogen, etc.) within the liquid phase of the multi-phase mixture in a defined range in the downward flow path
- **15** 114.

At **708** the temperature of the multi-phase mixture within the downward flow path 114 can be altered, adjusted, or controlled to maintain the temperature within a defined temperature range. In at least some instances, the defined temperature range may be selected or otherwise chosen based at

- 20 least in part on the microbiological species used within the fermentor **100.** Excess heat may be generated as a byproduct **by** the microbiological organisms responsible for at least a portion of the activity within the fermentor **100.** This excess heat, if left uncontrolled, can inhibit or adversely affect the growth or metabolism of some or all of the microbiological organisms within the
- **25** fermentor 100. In at least some instances, cooling may be provided within the downward flow path 114 to maintain the temperature of the multi-phase mixture in the downward flow path 114 within a defined range. Such cooling may include passage of a cooling media through reservoirs or coils 140 thermally conductively coupled to the downward flow path 114. Cooling water or other
- **30** cooling media (e.g., glycol solutions, brine solutions, etc.) may also be used to provide cooling within the fermentor **100.** In at least some instances, the control subsystem **290** may control the flow rate or temperature of the cooling media passed through the reservoirs or coils 140 that are thermally conductively coupled to the downward flow path **114.**
- **35** In other instances, the heat produced **by** the microbiological species may be insufficient to maintain the fermentor within a desired

temperature range. Such may occur, for example, in extremely cold environments where the fermentor **100** is located in an exposed or partially exposed exterior location. In some instances, the reservoirs or coils 140 thermally conductively coupled to the downward flow path 114 used for cooling

- **5** may also be used or provide warming to the fermentor **100.** In other instances, dedicated warming reservoirs or coils may be thermally conductively coupled to the downward flow path 114. Such warming may include passage of a warming media through the reservoirs or coils placed at least partially within the downward flow path 114. Warm water, steam, or similar heat transfer fluids
- **10** (e.g., glycol solutions, thermal oils, etc.) may also be used to optionally provide warming within the fermentor **100.** In at least some instances, the control subsystem **290** may control the flow rate or temperature of the warming media passed through the reservoirs or coils 140 that are thermally conductively coupled to the downward flow path **114.**
- **15** At **710,** the pressure on the gas substrate bubbles traveling with the multi-phase mixture in the downward flow **116** within the downward flow path **114** will increase as the gas substrate bubbles are pushed deeper along the downward flow path. The pressure increase may be at least partially attributable to the increase in hydrostatic pressure exerted on the gas substrate
- 20 bubbles **by** the column of liquid in the fermentor **100.** The pressure increase can, in some instances, advantageously increase the mass transfer between the gas substrate bubbles and the liquid media forming the multi-phase mixture in the downward flow path **114.** In at least some instances, the increased pressure on the gas substrate bubbles may also advantageously improve the
- **25** uptake of one or more components present in the gas substrate **by** the microbiological organisms present in the downward flow path.

At **712,** the multi-phase mixture exits the downward flow path and enters the upward flow path **118.** The multi-phase mixture entering the upward flow path can include, but is not limited to the liquid containing unabsorbed

- **30** nutrients, the gas substrate bubbles containing undissolved and unabsorbed gas substrate. The multi-phase mixture entering the upward flow path **118** may also contain entrained biological matter swept from the walls and/or floor of the fermentor **100.** In at least some instances, one or more structures promoting mass transfer may be disposed within at least a portion of the upward flow path
- **35 118.** Such structures may include, but are not limited to, one or more baffles, one or more unpowered or static mixers, one or more powered or dynamic

mixers, and the like. In at least some instances, additional liquid containing one or more nutrients or additional gas substrate may be introduced to the upward flow path **118.** Such additional nutrients or gas substrates may be introduced to replenish those consumed via chemical or microbiological processes in the

- **5** downward flow path **118.** Such additional nutrients or gas substrates may be introduced to provide additional or different nutrients or gas substrates to the microbiological organisms present in the upward flow path **118.** As the multi phase mixture flows upward 120, pressure on the gas substrate bubbles present in the multi-phase mixture will gradually lessen as the hydrostatic head
- **10** decreases on the rise through the upwards flow path **118.** At 714, the temperature of the multi-phase mixture within the

upward flow path **118** can be altered, adjusted, or controlled to maintain the temperature within a defined temperature range. In at least some instances, the defined temperature range may be selected or otherwise chosen based at

- **15** least in part on the microbiological species used within the fermentor **100.** Excess heat may be generated as a byproduct **by** the microbiological organisms responsible for at least a portion of the activity within the fermentor **100.** This excess heat, if left uncontrolled, can inhibit or adversely affect the growth or metabolism of some or all of the microbiological organisms within the
- 20 fermentor 100. In at least some instances, cooling may be provided within the upward flow path **118** to maintain the temperature of the multi-phase mixture in the upward flow path **118** within a defined range. Such cooling may include passage of a cooling media through reservoirs or coils **128** thermally conductively coupled to the upward flow path **118.** Cooling water or other
- **25** cooling media (e.g., glycol solutions, brine solutions, etc.) may also be used to provide cooling within the fermentor **100.** In at least some instances, the control subsystem **290** may control the flow rate or temperature of the cooling media passed through the reservoirs or coils **128** thermally conductively coupled to the upward flow path **118.**
- **30** In other instances, the heat produced **by** the microbiological species may be insufficient to maintain the fermentor within a desired temperature range. Such may occur, for example, in extremely cold environments where the fermentor **100** is located in an exposed or partially exposed exterior location. In some instances, the reservoirs or coils **128**
- **35** thermally conductively coupled to the upward flow path **118** used for cooling may also be used or provide warming to the fermentor **100.** In other instances,

dedicated warming reservoirs or coils may be thermally conductively coupled to the upward flow path **118.** Such warming may include passage of a warming media through the reservoirs or coils placed at least partially within the upward flow path **118.** Warm water, steam, or similar heat transfer fluids (e.g., glycol

5 solutions, thermal oils, etc.) may also be used to optionally provide warming within the fermentor **100.** In at least some instances, the control subsystem **290** may control the flow rate or temperature of the warming media passed through the reservoirs or coils **128** that are thermally conductively coupled to the upward flow path **118.** The process concludes at **716.**

- **10** Figure **8** shows a high level method of operation **800** of a fermentation system using one or more fermentors **100** in one or more fermentor systems **300,** 400, **500, 600** described in detail above with regard to Figures **1-6.** The example fermentation method **800** uses the identical or nearly identical steps to those described in detail with regard to the fermentation
- **15** method **700** discussed in detail in Figure **7,** with the exception that the fermentation method **800** is conducted at an elevated pressure using one or more backpressure subsystems **230.** Such backpressure subsystems **230** advantageously maintain the pressure in the fermentor **100** at a level greater than atmospheric pressure, thereby increasing the partial pressure of the
- 20 gas(es) contained in the gas bubbles **310** in the multi-phase mixture **312. By** increasing the partial pressure of the gas(es) within the gas bubbles **310,** the mass transfer rate between the gas bubbles **310** and the liquid **308** is enhanced and the increased availability of the dissolved gas(es) within the multi-phase mixture beneficially improves the uptake of the gas(es) **by** the microbiological
- **25** organisms in the fermentor **100.** The method commences at **802.** At 804 the liquid **308** and the gas(es) are introduced to the fermentor **100** at a first pressure that is greater than atmospheric pressure. The presence of the backpressure subsystem **230** maintains the pressure within the fermentor **100,** thereby increasing the pressure in both the downward flow path
- **30 114** and the upward flow path **118.** In at least some instances, the first pressure can be from about **5** psig to about **150** psig; from about **5** psig to about **100** psig; or from about **5** psig to about **75** psig. As the multi-phase mixture passes through the downward flow path 114, the hydrostatic pressure will build on the gas bubbles **310** further increasing the partial pressure of the gas(es)
- **35** present in the gas bubbles **310** and further increasing the mass transfer rate between the gas bubbles **310** and the multi-phase mixture. The pressure of the

multi-phase mixture will increase to a second pressure at the bottom of the downward flow path **118.** In at least some instances, the second pressure can be from about **10** psig to about **150** psig; from about **10** psig to about **100** psig; or from about **10** psig to about **75** psig. As the multi-phase mixture passes

- **5** through the upward flow path **118,** the hydrostatic pressure on the gas bubbles **310** will be relieved to a third pressure at the exit of the upward flow path **118.** In at least some instances, the third pressure can be from about **5** psig to about **150** psig; from about **5** psig to about **100** psig; or from about **5** psig to about **75** psig.
- **10** At **806** the multi-phase mixture exiting the hollow fluid conduits via the multi-phase mixture discharge fluid connection **138** is directed to the backpressure subsystem **230.** Within the backpressure subsystem **230,** one or more systems or devices are used to reduce the pressure of the multi-phase mixture to a level that is less than the third pressure. Such pressure reduction
- **15** may be accomplished using one or more pressure drop inducing devices such as an orifice plate or a backpressure control valve. In one or more instances, the one or more pressure drop inducing devices can include a multi-phase turbine through which the high pressure multi-phase mixture is passed to provide the low pressure multi-phase mixture **232** at a fourth pressure. In at
- 20 least some instances, the fourth pressure can be from about **5** psig to about **150** psig; from about **5** psig to about **100** psig; or from about **5** psig to about **75** psig. In at least some instances, at least a portion of energy released during the reduction in pressure of the multi-phase mixture **136** may be captured, for example the energy released in the multi-phase turbine may be used to turn an
- **25** electrical generator, fluid mover, or gas mover. The method concludes at **808.** Figure **9** shows a high level method of operation **900** of a fermentation system using one or more fermentors **100** in one or more fermentor systems **300,** 400, **500, 600** described in detail above with regard to Figures **1-6.** The example fermentation method **900** employs an identical or
- **30** nearly identical method to the fermentation methods **700** and **800** discussed in detail with regard to Figure **7** and Figure **8,** with the exception that the low pressure multi-phase mixture **232** removed from the backpressure subsystem **230** is introduced to and separated in a separation subsystem **250.** The separation subsystem **250** can include one or more devices or systems to
- **35** separate the low pressure multi-phase mixture into at least a gas and a liquid. In some instances, the separation subsystem **250** may separate the low

pressure multi-phase mixture **232** into a gas **252,** a liquid 254, and a solids-rich liquid **256.** The method commences at **902.**

At 904, the low pressure multi-phase mixture **232** from the separation subsystem **230** is introduced to the separation subsystem **250.**

- **5** Within the separation subsystem the multi-phase mixture **232** can be separated into at least a gas **252** and a liquid 254. In at least some instances, at least a portion of the separated gas **252** may be subsequently processed or separated. At least a portion of the processed or separated gas may be recycled to the fermentor **100** as a gas substrate 204. In some instances, at least a portion of
- **10** the separated gas may be sold or otherwise disposed. In at least some instances, at least a portion of the separated gas may be sold or traded as a fungible commodity. In at least some instances, the separated gas may include one or more **C2.** hydrocarbon gases, including but not limited to, ethane, ethylene, propane, butane, and compounds thereof.
- **15** In at least some instances, at least a portion of the separated liquid 254 may be subsequently processed or separated. At least a portion of the separated gas **252** may be subsequently processed or separated. At **906** at least a portion of the processed or separated liquid which may or may not include biosolids removed from the fermentor **100** with the multi-phase mixture
- 20 **136** can be recycled to the fermentor **100.** For example, at least a portion of the separated liquid **256** containing biosolids may be combined with at least a portion of the liquid feed 202 to provide the mixture **258** fed to the fermentor **100.** Such recycle may advantageously provide an ongoing, continuous or semi-continuous, inoculation of the fermentor **100** with established biological
- **25** species. In some instances, at least a portion of the separated liquid may be sold or otherwise disposed. In at least some instances, at least a portion of the separated liquid may be sold or traded as a fungible commodity. In at least some instances, the separated liquid may include one or more **C2.** hydrocarbon liquids, including but not limited to one or more alcohols, glycols, or ketones.
- **30** The process concludes at **908.**

The above description of illustrated embodiments, including what is described in the Abstract, is not intended to be exhaustive or to limit the embodiments to the precise forms disclosed. Although specific embodiments of and examples are described herein for illustrative purposes, various equivalent

35 modifications can be made without departing from the spirit and scope of the disclosure, as will be recognized **by** those skilled in the relevant art. The

teachings provided herein of the various embodiments can be applied to other fermentors and fermentation systems. Such fermentors and fermentation systems may include fermentors for purposes other than chemical intermediate production, and may include fermentors and fermentation systems useful in

- **5** food or beverage production. Similarly, the ancillary systems described herein, including the cooling subsystem, the backpressure subsystem, the separation subsystem, and the control subsystem may include a single system, for example a package cooling tower or package control system, or may include a custom designed subsystem including any number of subcomponents that are
- **10** physically, fluidly, and communicably coupled in a manner facilitating the controlled production and distribution of cooling or warming media (i.e., **by** the cooling subsystem); facilitating the controlled maintenance of backpressure on the fermentor system with or without the supplemental generation of energy (i.e., **by** the backpressure subsystem); facilitating the separation of at least a
- **15** portion of the multi-phase mixture into a gas, liquid, and semi-solid for recycle or for recovery and subsequent processing or sale (i.e., **by** the separation subsystem). The control subsystem can include an integrated or distributed control system that provides monitoring, alarming, control, and control output for all or a portion of the fermentation system or any of the ancillary
- 20 subsystems. The control subsystem may also include any number of individual loop controllers and the like for control of one or more aspects of the fermentation system or any of the ancillary subsystems.

The foregoing detailed description has set forth various embodiments of the devices and/or processes via the use of process flow **25** diagrams, equipment sectional views, and example methods. Insofar as such

- block diagrams, schematics, and examples contain one or more functions and/or operations, it will be understood **by** those skilled in the art that each function and/or operation within such block diagrams, flowcharts, or examples can be implemented, individually and/or collectively, using wide range of off
- **30** the-shelf or customized components that are well known to those of skill in the chemical engineering arts. For example, although only circular, triangular, and square hollow metal conduits are explicitly discussed, one of skill in the art would realize that virtually any hollow fluid conduit could be substituted. The microbiological species listed herein are intended to provide a sample of the
- **35** potential microbiological species that can be supported in a fermentor or fermentor system as described herein.

The disclosure of **U.S.** provisional patent application Serial No. 61/711,104, filed October **8,** 2012, is incorporated herein in its entirety.

The various embodiments described above can be combined to provide further embodiments. **All** of the **U.S.** patents, **U.S.** patent application

- **5** publications, **U.S.** patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, including but not limited to: **U.S.** Provisional Patent Application Serial No. **61/671,542,** filed July **13,** 2012; is incorporated herein **by** reference, in their entirety. Aspects of the embodiments can be
- **10** modified, if necessary, to employ systems, processes, biological media, and concepts of the various patents, applications and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the **15** terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of

equivalents to which such claims are entitled. Accordingly, the claims are not limited **by** the disclosure.

CLAIMS

1. A system to stimulate biological growth, the system comprising:

at least one hollow fluid conduit disposed within an interior space formed **by** a vessel, the at least one hollow fluid conduit permitting a fluid flow therethrough, the at least one hollow fluid conduit including at least one inlet and at least one outlet;

wherein the inlet of the at least one hollow fluid conduit is in fluid communication with the interior space of the vessel to provide a downward flow path bordered **by** an external perimeter of the at least one hollow fluid conduit and **by** an internal perimeter of the vessel and an upward flow path bordered **by** an internal perimeter of the at least one hollow fluid conduit;

at least one gas distributor disposed adjacent the at least one outlet of the at least one hollow fluid conduit and in the downward flow path to introduce one or more gases in at least a portion of the downward flow path;

at least one first fluid mover to provide a downward fluid flow in the downward flow path and an upward fluid flow in the upward flow path; and

a thermal transfer system thermally coupled to at least one of the downward flow path or the upward flow path.

2. The system of claim 1 wherein the at least one fluid mover is (a) disposed at least partially in the downward flow path and fluidly coupled to the at least one hollow fluid conduit inlet to induce a fluid flow in the downward flow path and to force a fluid flow in the upward flow path; **(b)** disposed external to both the upward flow path and the downward flow path, and is fluidly coupled to the downward flow path to force a fluid flow in both the upward flow path and the downward flow path; or (c) disposed external to both the upward flow path and the downward flow path, and is fluidly coupled to the upward flow path to induce a fluid flow in both the upward flow path and the downward flow path.

3. The system of claim 1 wherein the thermal transfer system thermally coupled to at least one of the downward flow path or the upward flow path comprises a thermal transfer system thermally conductively coupled to at least a portion of the at least one hollow fluid conduit or at least a portion of the vessel.

4. The system of claim 1 wherein the vessel further comprises a biomass accumulator to encourage an accumulation of excess biomass in at least a portion of the interior space formed **by** the vessel.

5. The system of claim 1 wherein at least a portion of the upward flow path includes (a) one or more structures to promote biomass growth or **(b)** one or more structures promoting mass transfer between phases of a multi-phase mixture flowing in the upward flow path.

6. The system of claim **1,** wherein at least a portion of the downward flow path includes (a) one or more structures to promote biomass growth, or **(b)** one or more structures promoting mass transfer between phases of a multi-phase mixture flowing in the downward flow path.

7. The system of claim **1,** further comprising at least one separator to promote the separation of a multi-phase mixture into a plurality of phases, the separator fluidly coupled to receive at least a portion of the multi-phase mixture from (a) the at least one hollow fluid conduit outlet.

8. The system of claim **7,** wherein the separator is further fluidly coupled to the vessel to return at least a portion of the separated multi-phase mixture to the downward flow path.

9. The system of claim **1,** wherein the at least one hollow fluid conduit includes (a) a single hollow fluid conduit having a longitudinal axis oriented substantially parallel to a longitudinal axis of the vessel; or **(b)** a plurality of hollow fluid conduits, each having a respective longitudinal axis that is oriented substantially parallel to a longitudinal axis of the vessel.

10. The system of claim 1 wherein the thermal transfer system includes a reservoir containing one or more heat transfer media thermally coupled to at least a portion of the vessel or at least a portion of the at least one hollow fluid conduit.

11. The system of claim 1 wherein an aggregate total transverse cross sectional area of the at least one hollow fluid conduit is at least **10%** of the total transverse cross sectional area of the vessel.

12. **A** method to stimulate biological growth, the method comprising:

dispersing a gas in a liquid media including at least water and one or more nutrients to form a multi-phase mixture comprising gas bubbles dispersed in the liquid media;

flowing at a first velocity and a first pressure the multi-phase mixture in one or more downward flow paths, before flowing the multi-phase mixture in a number of upward flow paths, the one or more downward flow paths formed between an exterior perimeter of a number of hollow fluid conduits and an interior perimeter of a vessel at least partially surrounding the number of hollow fluid conduits;

increasing the pressure of the multi-phase mixture from the first pressure to a second pressure to produce compressed gas bubbles in the multi-phase mixture while contacting the multi-phase mixture with a number of microorganisms existent in the downward flow path to provide a first biomass; and

flowing at a second velocity the multi-phase mixture in a number of upward flow paths, each of the respective number of upward flow paths formed **by** an interior perimeter of each of the number of hollow fluid conduits and maintaining the pressure at or above a third pressure to maintain compressed gas bubbles in the multi-phase mixture while contacting the multi-phase mixture with a number microorganisms existent in the upward flow path to provide a second biomass.

13. The method of claim 12, further comprising:

separating the multi-phase mixture removed from the one or more upward flow paths into a plurality of phases including at least a separated gas phase and a separated liquid phase.

14. The method of claim **13,** further comprising:

reducing the pressure of the multi-phase mixture removed from the one or more upward flow paths to a fourth pressure prior to separating the multi-phase mixture.

15. The method of claim 14, further comprising:

generating electricity **by** at least partially reducing the pressure of the multi-phase mixture to a fourth pressure using one or more energy capture systems.

16. An apparatus to stimulate biological growth, the apparatus comprising:

a number of hollow fluid conduits disposed at least partially within a vertically arranged vessel to provide a number of downward flow paths bordered **by** an exterior perimeter of each of the number of hollow fluid conduits and an interior perimeter of the vertically arranged vessel;

wherein the vertically arranged vessel includes at least one fluid inlet connection fluidly coupled to at least a portion of the number of downward flow paths;

wherein each of the number of hollow fluid conduits provides a respective number of upward flow paths, each of the upward flow paths bordered **by** an interior perimeter of each of the respective hollow fluid conduits, each of the number of upward flow paths fluidly coupled to at least one discharge connection to remove a multi-phase fluid;

at least one gas distributor disposed in at least a portion of the number of downward flow paths; and

a number of first structures disposed within at least a portion of at least one of: the number of downward flow paths or the upward flow paths, each of the number of first structures (a) to promote biological growth thereupon or **(b)** to promote mass transfer between phases of a multiphase mixture flowing in one of the number of downward flow path or upward flow path in which the number of first structures are disposed.

17. The apparatus of claim **16,** further comprising at least one thermal transfer surface disposed at least partially in the number of downward flow paths or at least partially in at least a portion of the number of upward flow paths.

18. The apparatus of claim **16** wherein an aggregate transverse cross-sectional area of the number of hollow fluid conduits is at least **10%** of the transverse cross-sectional area of the vessel.

19. The apparatus of claim **16** wherein the vessel further comprises a biomass accumulator to encourage an accumulation of excess biomass in at least a portion of the interior space formed **by** the vessel.

20. The apparatus of claim **16,** wherein the number of hollow fluid conduits includes (a) a single hollow fluid conduit having a longitudinal axis oriented substantially parallel to a longitudinal axis of the vessel; or **(b)** a plurality of hollow fluid conduits, each having a respective longitudinal axis that is oriented substantially parallel to a longitudinal axis of the vessel.

21. The method of claim 12 wherein contacting the multi-phase mixture with a number of microorganisms existent in the downward flow path to provide a first biomass comprises contacting the multi-phase mixture with (a) one or more structures to promote biomass growth or **(b)** one or more structures promoting mass transfer between phases of the multi-phase mixture flowing in the downward flow path.

22. The method of claim 12, wherein contacting the multi-phase mixture with a number of microorganisms existent in the upward flow path to provide a second biomass comprises contacting the multi-phase mixture with (a) one or more structures to promote biomass growth or **(b)** one or more structures promoting mass transfer between phases of the multi-phase mixture flowing in the upward flow path.

23. The system of claim **1,** further comprising at least one back pressure generator fluidly coupled to the outlet of the at least one hollow fluid conduit.

> **Calysta, Inc. Patent Attorneys for the Applicant/Nominated Person SPRUSON & FERGUSON**

 $FIG. 1$

 $6/9$

 $FIG. 7$

