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(71) Applicant: OAKBIO INC. [US/US]; 265 Sobrante Way, Suite T, Sunnyvale, California 94086 (US).

(72) Inventors: SEFTON, Brian; c/o Royse Law Firm, PC, 149 Commonwealth Drive, Suite 1001, Menlo Park, California 94025 (US). COLEMAN, William; c/o Royse Law Firm, PC, 149 Commonwealth Drive, Suite 1001, Menlo Park, California 94025 (US).

(74) Agent: HAYDEN, Robert; Royse Law Firm, PC, 149 Commonwealth Drive, Suite 1001, Menlo Park, California 94025 (US).

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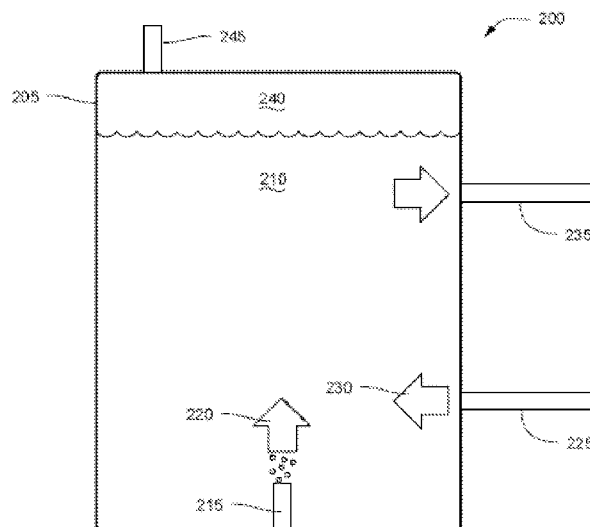


Figure 2

(57) Abstract: Aquafeed, animal feed, and other food products, as well as nutritional and pharmaceutical compounds, chemicals and biomaterials are important commodities that can be produced at commercial scale by fermentation of microorganisms. The present invention provides a method for producing these valuable multi-carbon compounds from simple gas feedstocks, such as carbon dioxide, hydrogen and oxygen, by cultivating a consortium of microbial cells specially selected for this purpose in an aqueous culture medium. In addition to exploiting inexpensive feedstocks, such as waste industrial gas for this cultivation, the platform described herein also provides the advantage of removing carbon dioxide and other waste gases from industrial emissions, which would otherwise contribute to global climate change. Furthermore, the cultivation of a microbial consortium can provide highly nutritious components to a feed blend that might not be available from a monoculture.

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Novel Microbial Biomass Based Feed Products

CROSS-REFERENCE TO RELATED APPLICATION

[001] This application claims the benefit of US Patent Application No. 15/641,114 filed on July 3, 2017 which is incorporated herein by reference.

BACKGROUND

[002] Field of the Invention

[003] The present invention is generally related to the fields of microbial fermentation and industrial biotechnology, including biological methods, processes and microorganisms for producing, via gas fermentation, nutritional products including high-protein food, food additives and other products from the gas streams which contain carbon and energy, which may be by-products, side products or products of various industrial processes.

[004] Related Art

[005] Gas feedstocks represent a vast resource of carbon and energy that can be used to grow chemoautotrophic microorganisms for use in human and animal nutritional products.

Chemoautotrophic bacteria are capable of capturing and metabolizing carbon from inorganic sources, such as CO₂, CO, CH₄, as well as, in some cases, hydrogen (H₂) or methane (CH₄) as a primary or additional source of energy. By converting inorganic carbon to organic carbon via metabolic carbon fixation, these microbes can serve as primary producers in natural environments. Many of these chemoautotrophic species can be cultured on gas feedstocks in bioreactors for commercial production of biomass, which can be processed into nutritional products such as animal feed, companion animal feed, or even food for humans, and chemicals.

[006] A microbial consortium is defined as two or more microbial species living symbiotically. Microbial co-cultures. For the consortia described as part of the present invention, chemoautotrophic microbes are employed in a system where the primary source of carbon and energy are both supplied as gases, such that the primary producers support a defined consortium of bacterial species via mutualistic interactions. The consortium of various defined species in the present invention, when harvested, can be used as a nutritional product. In order to make more ideal nutritional products, it is desirable to grow multiple different species together, each of which provides different nutritional properties. The use of multiple microbes allows controlled modification and customization of the nutritional composition of the final product. It is also possible to include one or more genetically modified strains which produce a key component, such as astaxanthin, a carotenoid which is a key ingredient for aquaculture.

[007] A formulation of bacteria that could increase the amounts of desirable fatty acids and amino acids in the final biomass product would therefore be highly desirable.

[008] An advantage of the present invention is that the dried biomass can be blended into an aquafeed or other nutritional product to replace the fishmeal that is normally harvested and used for feeding farmed fish and seafood, such as salmon, trout, tilapia, and shrimp. This will make a major impact on reducing the stress on the world's fisheries (Pitcher & Cheung, 2013), which will not be able to keep up with the projected demand for fishmeal.

[009] Another advantage of the present invention is that the composition of the consortium can be adjusted to fine-tune the nutritional composition of the biomass product. This ability to make adjustments is advantageous because, for example, recent studies have shown that adapting the amino acid composition of feed given to laboratory mice based on the animal's own overall amino acid composition not only reduces the amount of feed needed, but also improves the

health of the animal. Also, aquaculture and other animal feeds and feed additives are altered by selecting the amounts of various ingredients added to achieve desired amino acid profiles. By adjusting the consortia, a number of application specific nutritional products can be derived including for use by humans. Special consortia may even be created for aid in treating medical conditions, dietary deficiencies or to match growth stages of proposed consuming organism.

[0010] Nevertheless, bacteria comprising the genera *Bacillus*, *Lactobacillus*, *Bifidobacterium* and others, which are known to be of nutritional, and/or medicinal and/or probiotic benefit are not capable of growing on gas substrates, such as CO₂, H₂, CO and CH₄.

[0011] However, certain strains of chemoautotrophic microbes from the genera *Cupriavidus*, *Rhodobacter*, *Methylobacterium*, *Methylococcus*, *Rhodospirillum*, *Rhodopseudomonas*, *Rhodococcus*, and *Paracoccus* are known to grow on gaseous substrates, such as CO₂, H₂, CO and CH₄.

[0012] Photoautotrophic microbes are bacteria, cyanobacteria and algae that can fix carbon from CO₂ by utilizing light energy. However, certain species of photoautotrophic bacteria also exhibit chemoautotrophic metabolism, in that they are able to utilize hydrogen as an energy source to drive the fixation of CO₂ without light. Examples of these are the genera *Rhodobacter*, *Rhodospirillum* and *Rhodopseudomonas*. Many other such species are known in the literature. *C. necator* has been shown to grow faster and more efficiently on CO₂/H₂ than typical acetogens that are used in some anaerobic gas fermentation.

[0013] Bacteria grown under chemoautotrophic conditions express different amounts (and in some cases, different types) of proteins, enzymes, transporters, fats, oils, vitamins, co-factors and other biochemicals, than do those grown in traditional fermentation. Examples of this would be cytochromes, quinones (e.g., coenzyme Q), RuBisCO (Ribulose-1,5-bisphosphate carboxylase

oxygenase), polysaccharides, proteins as well as having overall different levels and ratios of amino acids, and different species, levels and ratios of fatty acids. Because of this, bacteria grown chemoautotrophically have different nutritional profiles than the same bacteria grown heterotrophically. These growth differences can be advantages for providing additional vitamins, minerals, cofactors, etc. to the biomass product.

[0014] Bacteria grown chemoautotrophically also have different secretory activity, which affects the content of the fermentation mix. Chemoautotrophic bacteria (and photoautotrophic bacteria grown chemoautotrophically) release chemicals into the growth medium, such as glycolates, polysaccharides, proteins (including one or more essential amino acids), amino acids, fats, oils, hydrocarbons, nucleic acids, organic acids, polyhydroxyalkanoates, phasins, carotenoids such as betacarotene and lutein, vitamins, gene transfer agents (GTA), polysaccharides, sugars, starches and other biomolecules that can then be utilized by non-autotrophic bacteria and other heterotrophic microorganisms as growth substrates and growth regulators. These non-autotrophic species include bacteria, cyanobacteria, algae, yeast and fungi. Certain microbes, such as algae, cyanobacteria and some bacteria which may be capable of growing autotrophically are also capable of growing heterotrophically and thus might also be grown this way.

[0015] In an alternative embodiment, one or more of the strains in the consortium may naturally, or be genetically modified in order to, produce a valuable molecule such as a specific fatty acid such as a polyunsaturated fatty acid, (eg: eicosapentaenoic acid and docosahexaenoic acid), vitamin, alcohol, sugar, ketone, ester, acid, di-acid, a colorant, fragrance, flavor, carotenoid, porphyrin, hormone, a polysaccharide, antibiotic or a protein product (e.g., an enzyme, therapeutic protein, antibiotic, hormone, vitamin, precursor, antibody or vaccine). The present

invention provides a means to produce such molecules using cheap gas feedstocks even if the host organism(s) cannot grow solely on gas.

[0016] In this application we describe an invention wherein a food or feed product with characteristics that provide nutritional, medical, and/or dietary benefits, and that comprises a consortium of chemoautotrophic microbes, photoautotrophic and non-chemoautotrophic microbes, is produced by cultivating these microbes on gaseous substrates. The consortium creates an ecosystem in which the chemoautotrophic microbes form the base of a food chain on which the non-autotrophic microbes are capable of growing. The goal of the gas-based fermentation process described herein is to generate a biomass product that has enhanced nutritional value compared to the biomass that the same chemoautotrophic microbes would produce if grown alone. A further economic benefit of this invention is to facilitate the growth of desired or beneficial microbes that otherwise could not utilize the inexpensive CO₂ or other C1 feedstocks as a primary carbon source, and/or the hydrogen (or other inorganic or C1 compounds) for energy.

[0017] A number of species of microbe are known to nutritionally beneficial, or to produce beneficial substances, or have probiotic properties which can be significant components of a product which can be produced by this method. Examples of these are: *Arthiropsira platensis*, *Aspergillus niger*, *Aspergillus oryzae*, *Bacillus coagulans*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus subtilis*, *Bacteroides amylophilus*, *Bacteroides capillosus*, *Bacteroides ruminicola*, *Bacteroides suis*, *Bifidobacterium adolescentis*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium thermophilum*, *Bifidobacterium breve*, *Brevibacterium linens*, *Chlorella emersonii*, *Chlorella protothecoides*, *Chlorella pyrenoidosa*,

Chlorella vulgaris, *Dunaliella salina*, *Haematococcus pluvialis*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus cellobiosus*, *Lactobacillus curvatus*, *Lactobacillus delbruekii*, *Lactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus johnsonii*, *Lactobacillus lactis*, *Lactobacillus paracasei*, *Lactobacillus parafarraginis*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactobacillus sporogenes*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Nannochloropsis gaditana*, *Nannochloropsis granulata*, *Nannochloropsis limnetica*, *Nannochloropsis oceanica*, *Nannochloropsis oculata*, *Nannochloropsis salina*, *Paracoccus zeaxanthinifaciens*, *Paracoccus carotinifaciens*, *Paracoccus marcusii*, *Paracoccus sp.* *Pediococcus acidilactici*, *Pediococcus cerevisiae*, *Pediococcus pentosaceus*, *Propionibacterium shermanii*, *Propionibacterium freudenreichii*, *Rhodotorula glutinis*, *Saccharomyces boulardii*, *Saccharomyces cerevisiae*, *Streptococcus cremoris*, *Streptococcus diacetylactis*, *Streptococcus faecium*, *Streptococcus intermedius*, *Streptococcus lactis*, *Streptococcus thermophiles*, *Xanthophyllomyces dendrorhous*.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. 1 is a bright field micrograph of a consortium grown on CO₂, H₂ and O₂, which shows the multiple species of microbes, according to various embodiments.

[0019] FIG. 2 is a schematic representation of a bioreactor according to an exemplary embodiment of the present invention, according to various embodiments.

SUMMARY

[0020] In accordance with the present invention, products, materials, intermediates, and the like such as protein biomass and/or other biological products are produced from the waste gases of industrial processes by cultivating a microbial consortium. Such gases may include CO₂, CO, CH₄, and H₂, thereby reducing environmental pollution while at the same time saving energy and chemical feedstocks. Other trace gases which may be present in the industrial effluent, such as H₂S or SO₂, can provide additional nourishment to the autotrophic primary producer bacteria, and removal of such gases provides the added benefit of remediating these toxic GHGs from the gas stream.

[0021] In accordance with an exemplary process of the present invention, the desired components of the gas mixtures are introduced into a bioreactor containing one or more cultured strains of microbes that utilize the waste gas components to produce a desired compound. The desired product (biomass, nutraceutical, protein, etc.) is recovered from the aqueous phase in a separate vessel or vessels, utilizing a suitable recovery process for the compound produced. Examples of recovery processes include extraction, distillation or combinations thereof, or other efficient recovery processes. The bacteria are removed from the aqueous phase and recycled to avoid toxicity and maintain high cell concentrations, thus maximizing reaction rates. Cell separation, if desired, is accomplished by centrifugation, membranous ultrafiltration, or other techniques.

[0022] The principal object of the present invention is the provision of a process and/or a consortium of microorganisms for the production of products, intermediates, materials, and the like such as biomass, feed ingredients, proteins, vitamins, probiotics, natural antibiotics, organic acids, and the like from carbon dioxide, hydrogen, and oxygen.

[0023] Another object of the present invention is the provision of methods, microorganisms and apparatus for the production of items such as biomass, feed ingredients, proteins, vitamins, probiotics, natural antibiotics, and organic acids from the waste gas streams of industrial processes, such as brewing, bioethanol production, cement manufacturing, oil refining, and similar processes that generate waste CO₂ and/or H₂.

[0024] Yet another object of the present invention is the provision of a method, microorganism and apparatus involving continuous gaseous substrate fermentation under aerobic conditions to accomplish the conversion of waste gas streams of certain industrial processes into useful products such as biomass, feed ingredients, proteins, vitamins, probiotics, natural antibiotics, and organic acids.

[0025] Other objects and further scope of the applicability of the present invention will become apparent from the detailed description to follow, taken in conjunction with the accompanying drawings wherein like parts are designated by like reference numerals.

DETAILED DESCRIPTION

[0026] **Microbial strains.** *C. necator* has the advantage that it can grow very rapidly and to high density on a mixture of H₂, CO₂, O₂, and it can be continuously cultured for long periods of time without contamination. The same is true for *C. basilensis*, and for *Methylococcus capsulatus* where CH₄ is added or used in place of H₂. Bacterial strains for the consortium are selected for being both naturally-occurring (i.e., non-GMO), generally recognized as safe ('GRAS') organisms, or because of their apparent beneficial qualities and apparent lack of negative characteristics, so that they will be broadly suitable for feed and food processing, although GMO organisms designed for a specific purpose (e.g., metabolite production via an

engineered pathway) can also be included, if desired. All strains for the invention described herein were obtained as pure, axenic type-cultures from culture collections.

[0027] Gas supply. The CO₂, H₂ and O₂ can be supplied from either flue gas collected from an industrial emitter (designated as 'flue gas') or from pure stocks of compressed gas obtained from a gas supplier (designated as 'lab gas'), from process gas produced by an industrial process, from gasifier or pyrolysis output gas, syngas, from the manufacture of cement, from a combustion process, or from any industrial, natural or other process which produces one or more of the desired gases. For production of feed, food, nutraceuticals, biologicals, and the like, an industrial source of waste CO₂ that is free of toxic elemental contaminants (*e.g.*, mercury) is preferred. Examples of such sources include CO₂ from breweries and bioethanol plants. Hydrogen can be supplied as part of the gas composition of pyrolysis gas, syngas, as an industrial side product from activities such as propylene manufacture, as a component of a mixed gas stream from an oil refinery, or in gas created by steam methane reformation (SMR) process), from compressed gas, or from electrolysis of water. Oxygen can be obtained from atmospheric gas, as a product of electrolysis, or as a component of industrial by-product gas such as cement flue-gas. Embodiments of the invention include methods for collecting and compressing flue gas at an industrial site into transportable pressurized cylinders so that substantial quantities of flue gas can be carried back to the laboratory for analysis and fermentation of the microorganisms. In the laboratory, the CO₂ and O₂ (derived from either lab gas or flue gas) are further diluted approximately 5-fold with H₂ to supply the bacteria with a feedstock mixture that is optimized for growth. For a commercial-scale operation, the fermentation plant can be located near the gas production site, or the gas can be transported by vehicle or pipeline to the biomass production site.

[0028] For injection into the fermenter, the gas supply was filtered through 0.2 um filters to remove particles and microorganisms. For small-scale experiments, compressed H₂, CO₂ and O₂ were each regulated to 20 psi. The gases were delivered to a flow proportioner, which sets the relative fraction of the gases, and a variable area flow meter to control the mixture and flow rate into the fermenter. Gas flow was adjusted to between 0.2-1.2 VVM to supply adequate nutrients at each stage of the fermentation. The agitation rate was adjusted between 150-300 rpm to provide thorough mixing.

[0029] Nutrient monitoring. The composition of the input and output gas can be measured and monitored to determine the gas uptake rates, the mass balances and the mass transfer efficiency for dissolution of the gas into the solution and the biomass. Key nutrients (such as NH₄, PO₄ & SO₄), can also be monitored and replenished to prevent nutrient limitations that might restrict bacterial growth.

[0030] Microbial inocula. The inocula for fermenter runs can be prepared in many ways; each microbial strain may be grown separately, or two or more may be grouped together in a single fermentation. Heterotrophic species are always grown up from pure cultures on heterotrophic medium that is suitable for propagating the particular species (or group of species) being grown. Chemoautotrophic species can be grown on gases, or, in some cases, on heterotrophic media. Photoautotrophic species may be grown using light or heterotrophic media. Some photoautotrophic species are also chemoautotrophic, and thus may be grown on gaseous substrates. Inoculating the bioreactor involves sterile addition of a culture containing one or more of the species into the bioreactor.

[0031] In some embodiments, all of the cultures for the consortium are added to the bioreactor, in a short period of time, at the beginning of the fermentation procedure or run.

[0032] In some embodiments, chemoautotrophic microbes are added to the bioreactor at the beginning of the fermentation, and the inoculum cultures containing other species are added at later points.

[0033] In some embodiments, the timing of the addition, amount and density of culture additions, and method of preparing inocula can be altered to affect the qualities, composition, and/or value of the final product.

[0034] In some embodiments, additional inoculations of one or more strains used in the consortia can be added at later times.

[0035] In embodiments of the invention, cultures were prepared by growing *C. necator* and the other chemoautotrophic species on H₂/CO₂/O₂ to an OD₆₂₀ ~1 in small bottles of media equipped with gas fittings. Non-chemoautotrophic species were grown in liquid yeast-tryptone medium (YT medium), a well-known and commercially available medium. The bioreactor was inoculated to OD ~0.1. A ca. 5% inoculum is ideal. The pH is controlled with 2N NH₄OH. The fermentation is run for up to several days, resulting in OD₆₂₀ of 1-100 or greater. The recovered biomass was analyzed for protein and lipid content and the composition of each product.

Proprietary strains, of some embodiments of the invention, of *C. necator* and/or *R. capsulatus*, or other proprietary strains of microbe, were sometimes used in addition to type strains.

Embodiments of the invention include several strains of chemoautotrophic species that are adapted to flue gas and therefore tolerant to various toxic gas components, which can be included in the mix if complex industrial flue gas is used as the feedstock. In some cases, additional inoculations with one or more consortia strains were carried out at later time points.

[0036] Microbe species commonly used in various embodiments for enablement include those shown in Table 1: The micrograph in Figure 1 is from a fermentation of all of these except B-3226.

Strain	Species	Source
B-3226	<i>Rhodospirillum rubrum</i> , (ARS NRRL Type Strain)	
B-1727	<i>Rhodobacter sphaeroides</i> , (ARS NRRL Type Strain)	
B-4276	<i>Rhodopseudomonas palustris</i> , (ARS NRRL Type Strain)	
B-14308	<i>Bacillus megaterium</i> , (ARS NRRL Type Strain)	
B-356	<i>Bacillus subtilis</i> , (ARS NRRL Type Strain)	
B-354	<i>Bacillus subtilis</i> , (ARS NRRL Type Strain)	
B-14200	<i>Bacillus subtilis</i> subspecies <i>subtilis</i> , (ARS NRRL Type Strain)	
B-41406	<i>Bifidobacterium animalis</i> subspecies <i>animalis</i> , (ARS NRRL Type Strain)	
B-4495	<i>Lactobacillus acidophilus</i> , (ARS NRRL Type Strain)	
B-1922	<i>Lactobacillus casei</i> subspecies <i>casei</i> , (ARS NRRL Type Strain)	
B-4383	<i>Cupriavidus necator</i> , (ARS NRRL Type Strain)	
B-14690	<i>Cupriavidus necator</i> , (ARS NRRL Type Strain)	
	<i>Cupriavidus necator</i> strain H16 (ATCC Type Strain)	
	<i>Rhodobacter capsulatus</i> strain SB-1003 (ATCC Type Strain)	
OB213	<i>Rhodobacter capsulatus</i> , Oakbio, Inc. Proprietary Strain	
OB311	<i>Cupriavidus necator</i> , Oakbio, Inc. Proprietary Strain	

[001] Table 1.

[002] Bioreactor Fermentation. A bioreactor for chemoautotrophic synthesis is used for enablement of this invention. Many types and designs of bioreactor are suitable. The critical parts of a bioreactor for cultivation of the product discussed in this application are that there be a vessel which is at least partially filled with liquid medium, in which the microbes are dispersed.

The liquid comprises chemicals required for growth of the microbes, examples of which are described below. At least one port exists for introducing the gaseous substrates into the liquid in the bioreactor. The vessel may have a headspace into which gases collect after traversing the fluid in the vessel. An exhaust port allows gases to exit the vessel. Additional ports are present as needed for sensors, addition of liquids or chemicals and removal of product, liquids, or samples for testing, as would be expected to be found on common bioreactors, which are well known in the field of fermentation, cell culture and microbe cultivation. A minimal design bioreactor is shown in Figure 2. In various embodiments, the invention has been enabled in custom-built 250ml, 1L, and 4L glass flask based bioreactors and in a commercially manufactured New Brunswick Scientific Bio Flo 4500 with the 4-gas handling option. Additional bioreactor designs that can be used in conjunction with the present invention can be found in U.S. Patent Application Serial No. 13/204,649 filed on August 6, 2011 and entitled “Chemoautotrophic Bioreactor Systems and Methods of Use” which is incorporated herein by reference. Bioreactors for embodiment of this invention can comprise one or more vessels and/or towers or piping arrangements, and can comprise, for example, a Continuous Stirred Tank Reactor (CSTR), an Immobilized Cell Reactor (ICR), a Trickle Bed Reactor (TBR), a Bubble Column, a Gas Lift Fermenter, a Static Mixer, a Fluidized Bed, an Up-flow or Down-flow, a continuous, batch or loop reactor, or any other vessel or device suitable for maintaining suitable gas-liquid contact. In some embodiments, the bioreactor may comprise a first growth vessel and a second chemoautotrophic synthesis vessel, while in other embodiments a single vessel is used throughout both of the growth and synthesis stages.

[003] In some embodiments, a gas recirculation system can be used to improve the conversion efficiency, particularly during a continuous process, in order to reduce the total gas

requirement. Continuous harvesting of the cell mass is advantageous for a commercial production process, and can be implemented through the continuous removal of cell broth and the continuous replenishing of medium, in order to maintain the culture volume and cell density. Fermentations were run at a constant or varied temperatures between 15 and 70 C, but the preferred temperature is 30 C.

[004] Monitoring cell growth and species diversity. To monitor the progress of cell growth and verify the species diversity of the culture, samples can be periodically removed for analysis, or the bioreactor system can comprise analytic equipment. In enabling the technology in some embodiments, characterization included microscopy of cell morphology, an example of which is shown in Figure 1 (from the completion of a run), which shows that the species diversity was maintained. Wet mounts of the culture were observed using brightfield microscopy with an Olympus BX research microscope equipped with an Amscope CCD camera. Micrographs were generated using the Amscope software for imaging and data storage. Species diversity can also be monitored and quantitated using methods well known in the art, such as analysis of 16S rRNA genes, 23S rRNA genes, or other genetic markers and phenotypic indicators (Jovel et al., 2016). Growth behavior was characterized by optical density (OD) measurements at 620 nm using an ICN TiterTek 96-well plate reader. Aliquots of each fermenter sample (200 ul) were measured in duplicate to plot the growth curves.

[005] Carbon capture. Carbon capture from a new source of flue gas can be verified by performing headspace gas analysis, as well as growth experiments that use the flue gas as the sole carbon source for bacterial biomass production. The dry weight of each culture can also be determined by centrifuging the culture, washing the pellet, drying the cells in a lyophilizer, and weighing the lyophilized cells.

[006] Gas mixing. For hydrogen fermentations typically, the CO₂ feedstock or raw flue gas was diluted with pure hydrogen with ratios of about 8:1 to 1:1 (H₂:CO₂, v/v), resulting in a final CO₂ concentration of about 50% - 1% or less. The O₂ concentration is ideally 3-12%. For methane fermentations, typically the methane concentration is between 80% to 5%, CO₂ is between 40% - 1%, and oxygen is 50% to 5%. In either system, CO can be up to 10%, and a variety of other gases may be present, including sulfur oxides, nitrogen oxides, hydrogen sulfide, molecular nitrogen or other gases found in the gas source.

[007] Culture medium. Many different mineral media recipes can be used, and varying the media is one of the ways the characteristics of the final product can be influenced. In various embodiments, typically a mineral salts medium (modified from Repaske & Mayer, 1976) was used that contained no organic carbon or complex nutrients: Na₂HPO₄·2H₂O 4.5 g/L, KH₂PO₄ 1.5 g/L, NH₄Cl 1.8 g/L, MgSO₄·7H₂O 0.11 g/L, NaHCO₃ 0.2g/L, FeSO₄·7H₂O 12 mg/L, CaCl₂·2H₂O 10 mg/L, ZnSO₄·7H₂O 100 µg/L, MnCl₂·4 H₂O 30 µg/L, H₃BO₃ 300 µg/L, CoCl₂·6H₂O 200 µg/L, CuCl₂·2H₂O 10 µg/L, NiCl₂·6H₂O 20 µg/L, Na₂MoO₄·2H₂O 30 µg/L.

[008] Concentration and harvesting. Biomass product can be harvested through many methods, such as filtration, gravity separation, or other method, of which many are industrially practiced. Drying can be by spray drying, freeze drying, thermal drying, desiccation or many other methods, many of which are currently practiced industrially.

[009] Brief heat treatment is useful if the cells must be made non-viable prior to further processing. Due to the fact that lyophilization is more energy-intensive, it is more suitable for processing very high-value products that require gentle processing. The dried material can be easily blended with other ingredients to form a nutritious fish feed that can replace aquafeed products that typically rely on fishmeal for protein, fatty acids, and other nutrients. The amino

acid composition of the dried material from a 30L batch of cultivated consortium (Table 2) compares favorably to that of fishmeal (IAFMM Report, 1970).

[0010] In the below referenced demonstration, cell suspensions were removed from the fermenter via the sterile exit port. The supernatant can then be removed by centrifugation in a standard or process centrifuge at ca. 4,000 x g or greater to form a cell pellet. The cells are then washed in a low-salt buffer solution, and then re-pelleted. The final cell paste was then freeze dried to a powder using a commercial MTS lyophilizer.

[0011] Table 2. An example of the amino acid composition of a consortium. A sample of the consortia grown, in various embodiments, consisting of chemoautotrophs, photoautotrophs, and probiotic heterotrophs, cultivated at the 30L scale in a New Brunswick Scientific BioFlo 4500 Bioreactor with 4-gas input and control option. Amino acid composition analysis was conducted by NP Analytical Laboratories (St. Louis, MO).

Amino Acid	g/100 g Dried Biomass
Aspartic Acid	4.49
Threonine	2.40
Serine	1.79
Glutamic Acid	7.31
Proline	1.67
Glycine	2.15
Alanine	3.24
Valine	3.34
Methionine	1.16
Isoleucine	2.60

Leucine	3.24
Tyrosine	1.71
Phenylalanine	1.99
Histidine	0.930
Lysine	3.48
Arginine	2.45
Cysteine	0.312
Tryptophan	0.519

[0012] PATENT CITATIONS

Cited Patent	Filing Date	Publication Date	Applicant	Title
US 9267158 B2	Jan 7, 2016	Jul 26, 2016	Intrexon Corp.	Biological production of multi-carbon compounds from methane.
US7579163 B2	Aug 16, 2002	Aug 25, 2009	Statoil Asa	Method of fermentation
US 6340581 B1	Dec 23, 1998	Jan 22, 2002	Bioengineering Resources, Inc.	Biological production of products from waste gases
US 9206451 B2	Sep 11, 2012	Dec 8, 2015	Oakbio, Inc.	Chemoautotrophic conversion of carbon oxides in industrial waste to biomass and chemical products

EP 1419234 A1	Aug 16, 2002	May 19, 2004	Cockbain, Julian, Norferm DA	Method of fermentation
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[0013] FIG. 1 shows a bright field micrograph of a consortium grown on CO₂, H₂ and O₂, taken 5/15/2017. This picture shows a mix of chemoautotrophic and heterotrophic microbes grown with CO₂ as a primary carbon source and H₂ as a primary energy source. A short, thin rod 100, a medium-length, fat rod 110 (characteristic of *C. necator*), a long rod 120 (characteristic of *B. megaterium*), and a coccus 130 are indicated by arrows.

[0014] FIG. 2 shows a schematic representation of a bioreactor 200 as one example of a

[0015] bioreactor that is suitable for culturing a consortium on gas. Bioreactor 200 can comprise either a synthesis vessel for use in conjunction with a separate growth vessel, or can comprise a vessel suitable for both of the growth and synthesis stages. In FIG. 2, bioreactor 200 includes a vessel 205 that in operation holds a quantity of a liquid medium 210 containing the chemoautotrophic micro-organisms and other microbes in culture. The bioreactor 200 also includes a substrate port 215 through which a gaseous substrate 220 can be introduced into the vessel 205 for introduction into the liquid medium 210, a media inlet port 225 through which fresh media 230 can be introduced into the vessel 205 for introduction into the liquid medium 210, and a media outlet port 235 through which the medium 210 can be removed, for example, to harvest biomass and/or chemical products. The bioreactor 200 can also comprise a headspace 240 and a gas release valve 245 to vent gases from the headspace 240. In some embodiments, the media outlet port 235 and the media inlet port 225 are connected via a system which harvests biomass 170 and reconditions the medium 210 for recirculation. In some embodiments, the gas

release valve 245 is attached to a system which re-circulates the gaseous substrate back to the substrate port 215, and may make additions or subtractions to optimize the gas composition.

CLAIMS

What is claimed is:

1. A food and/or feed additive product comprising:

a consortium of chemoautotrophic and photoautotrophic bacteria and heterotrophic

microbes, the consortium including

chemoautotrophic bacteria from the genera *Cupriavidus*, *Rhodococcus* or

Methylococcus,

photoautotrophic bacteria from the genera *Rhodobacter*, *Rhodospirillum*,

Rhodopseudomonas, or *Arthrospira*, and

heterotrophic microbes from the genera *Bacillus*, *Bacteroides*, *Bifidobacterium*,

Lactobacillus, *Lactococcus*, *Leuconostoc*, *Paracoccus*, *Pediococcus*,

Acidilactici, *Pediococcus*, *Propionibacterium* or *Streptococcus*.

2. A method comprising:

providing a consortium of chemoautotrophic and photoautotrophic bacteria and

heterotrophic microbes within a system, the system including a fermentation

vessel filled with an aqueous medium comprising the consortium, the

fermentation vessel having an input port into which gaseous substrates are

introduced into the aqueous medium, the fermentation vessel further having an

exhaust port through which gases exit the fermentation vessel, wherein the

aqueous medium comprises inorganic anions and inorganic cations, and wherein

the aqueous medium contains a concentration of no more than 0.05% of each of

added sugars, yeast extract, tryptone, and/or organic carbon compounds, the consortium including chemoautotrophic bacteria from the genera *Cupriavidus*, *Rhodococcus* or *Methylococcus*, photoautotrophic bacteria from the genera *Rhodobacter*, *Rhodospirillum*, *Rhodopseudomonas*, or *Arthrospira*, and heterotrophic microbes from the genera of *Bacillus*, *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Paracoccus*, *Acidilactici*, *Pediococcus*, *Propionibacterium*, or *Streptococcus*;

introducing a first gaseous substrate into the input port, the first gaseous substrate including carbon predominantly in the form of single-carbon atom molecules;

introducing a second gaseous substrate into the aqueous medium, the second gaseous substrate including predominantly hydrogen gas (H₂) or methane gas (CH₄) or a mixture thereof, wherein either of the first or second substrates additionally includes oxygen gas (O₂); and

harvesting cells of the bacteria from the system.

3. A product made by the method of claim 2.

4. The method of claim 2 or product of claim 3, wherein the inorganic anions comprise phosphate, nitrate, sulfate, carbonate, and/or ammonium.

5. The method or product of claim 2, 3, or 4, wherein the inorganic cations comprise iron, nickel, calcium, magnesium, manganese, and/or cobalt.
6. The method or product of claims 1-4 or 5, wherein
- the chemoautotrophic bacteria include one or more of *Cupriavidus necator*, *Cupriavidus basilensis*, *Methylococcus capsulatus*, and *Rhodococcus opacus*,
- the photoautotrophic bacteria include one or more of *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, *Rhodobacter sphaeroides*, *Rhodopseudomonas palustris*, and *Arthiropira platensis*, and
- the heterotrophic microbes include one or more of *Arthiropira platensis*, *Aspergillus niger*, *Aspergillus oryzae*, *Bacillus coagulans*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus subtilis*, *Bacteroides amylophilus*, *Bacteroides capillosus*, *Bacteroides ruminicola*, *Bacteroides suis*, *Bifidobacterium adolescentis*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium thermophilum*, *Bifidobacterium breve*, *Brevibacterium linens*, *Chlorella emersonii*, *Chlorella protothecoides*, *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Dunaliella salina*, *Haematococcus pluvialis*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus cellobiosus*, *Lactobacillus curvatus*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus johnsonii*, *Lactobacillus lactis*, *Lactobacillus paracasei*, *Lactobacillus parafarraginis*, *Lactobacillus plantarum*, *Lactobacillus reuterii*,

Lactobacillus rhamnosus, *Lactobacillus salivarius*, *Lactobacillus sporogenes*,
Lactococcus lactis, *Leuconostoc mesenteroides*, *Nannochloropsis gaditana*,
Nannochloropsis granulata, *Nannochloropsis limnetica*, *Nannochloropsis oceanica*,
Nannochloropsis oculata, *Nannochloropsis salina*, *Paracoccus zeaxanthinifaciens*,
Paracoccus carotinifaciens, *Paracoccus marcusii*, *Paracoccus* sp. *Pediococcus*
acidilactici, *Pediococcus cerevisiae*, *Pediococcus pentosaceus*,
Propionibacterium shermanii, *Propionibacterium freudenreichii*, *Rhodotorula*
glutinis, *Saccharomyces boulardii*, *Saccharomyces cerevisiae*, *Streptococcus*
cremoris, *Streptococcus diacetylactis*, *Streptococcus faecium*, *Streptococcus*
intermedius, *Streptococcus lactis*, *Streptococcus thermophiles*,
Xanthophyllomyces dendrorhous.

7. The method or product of claims 1-5 or 6, wherein the chemoautotrophic bacteria comprise

Cupriavidus necator.

8. The method or product of claims 1-6 or 7, wherein the photoautotrophic bacteria comprise

Rhodobacter capsulatus.

9. The method or product of claims 1-7 or 8, wherein the heterotrophic microbes comprise

Bifidobacterium animalis.

10. The method or product of claims 1-8 or 9, wherein the chemoautotrophic bacteria comprise

Rhodococcus opacus.

11. The method or product of claims 1-9 or 10, wherein the heterotrophic microbes comprise

Lactobacillus acidophilus and/or *Lactobacillus casei*.

12. The method or product of claims 1-10 or 11, wherein the heterotrophic microbes comprise

Bacillus megaterium.

13. The method or product of claims 1-11 or 12, wherein heterotrophic microbes comprise a yeast or fungus.

14. The method or product of claims 1-12 or 13, wherein providing the consortium of chemoautotrophic, photoautotrophic, and heterotrophic microbes includes adding the chemoautotrophic bacteria into the fermentation vessel and then adding photoautotrophic and heterotrophic microbes to the fermentation vessel.

15. The method or product of claims 1-13 or 14, wherein providing the consortium of chemoautotrophic, photoautotrophic, and heterotrophic microbes includes adding the chemoautotrophic, photoautotrophic, and heterotrophic microbes into the fermentation vessel simultaneously.

16. The method or product of claims 1-14 or 15, wherein a concentration of single-carbon atom molecules in the first gaseous substrate and a concentration of hydrogen gas in the second gaseous substrate are both higher concentrations than are found in the ambient atmosphere.

17. The method or product of claims 1-14 or 15, wherein the single-carbon atom molecules comprise CO₂ and the first gaseous substrate includes a concentration of CO₂ and a concentration of CH₄, wherein the second substrate includes a concentration of hydrogen gas, and wherein all three concentrations are higher concentrations than are found in the ambient atmosphere.
18. The method or product of claims 1-14 or 15, wherein the single-carbon atom molecules comprise CO, CO₂, and CH₄, and the first gaseous substrate includes a concentration of CO₂, a concentration of CH₄, and a concentration of CO, wherein the second substrate includes a concentration of hydrogen gas, and wherein all four concentrations are higher concentrations than are found in the ambient atmosphere.
19. The method or product of claims 1-14 or 15, wherein the single-carbon atom molecules comprise CO and CH₄ and the first gaseous substrate includes a concentration of CO and a concentration of CH₄, wherein the second substrate includes a concentration of hydrogen gas, and wherein all three concentrations are higher concentrations than are found in the ambient atmosphere.
20. The method or product of claims 1-14 or 15, wherein the single-carbon atom molecules comprise CH₄, CO, and CO₂.

21. The method or product of claims 1-14 or 15, wherein the single-carbon atom molecules comprise CO and CH₄ and the first gaseous substrate includes a concentration of CO and a concentration of CH₄, wherein the second substrate includes a concentration of hydrogen gas, and wherein all three concentrations are higher concentrations than are found in the ambient atmosphere.
22. The method or product of claims 1-14 or 15, wherein the single-carbon atom molecules comprise CO and CH₄ and the first gaseous substrate includes a concentration of CO and a concentration of CH₄, and wherein both concentrations are higher concentrations than are found in the ambient atmosphere.
23. The method or product of claims 1-21 or 22, wherein one of the bacteria has been genetically modified.
24. The method or product of claims 1-22 or 23, wherein one of the bacteria of the consortium produces a carotenoid.
25. The method or product of claims 1-23 or 24, wherein one of the bacteria of the consortium produces a lutein.
26. The method or product of claims 1-24 or 25, wherein one of the bacteria of the consortium produces a vitamin.

27. The method or product of claims 1-25 or 26, wherein one of the bacteria of the consortium produces a protein.
28. The method or product of claims 1-26 or 27, wherein one of the bacteria of the consortium produces an astaxanthin.
29. The method or product of claims 1-27 or 28, wherein one of the bacteria of the consortium produces a polyunsaturated fatty acid.
30. The method or product of claims 1-28 or 29, wherein one of the bacteria of the consortium produces an eicosapentaenoic acid.
31. The method or product of claims 1-29 or 30, wherein one of the bacteria of the consortium produces a docosahexaenoic acid.
32. The method or product of claims 1-30 or 31, wherein one of the bacteria of the consortium produces a protein including an essential amino acid.
33. The method or product of claim 13 where yeast or fungi comprises a species of the genera, *Aspergillus*, *Saccharomyces*, *Xanthophyllomyces*, or *Rhodotorula*.

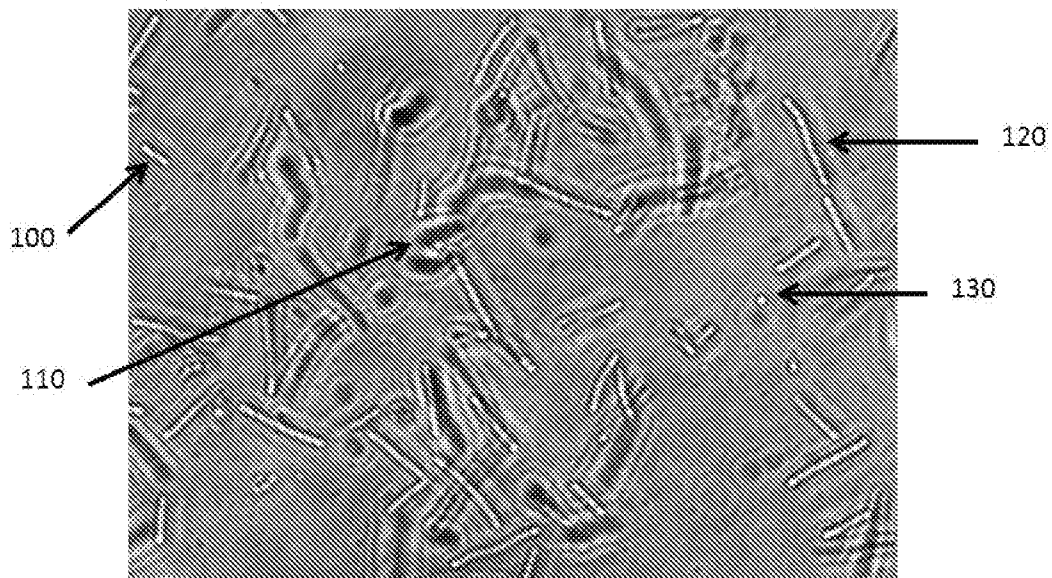


Figure 1

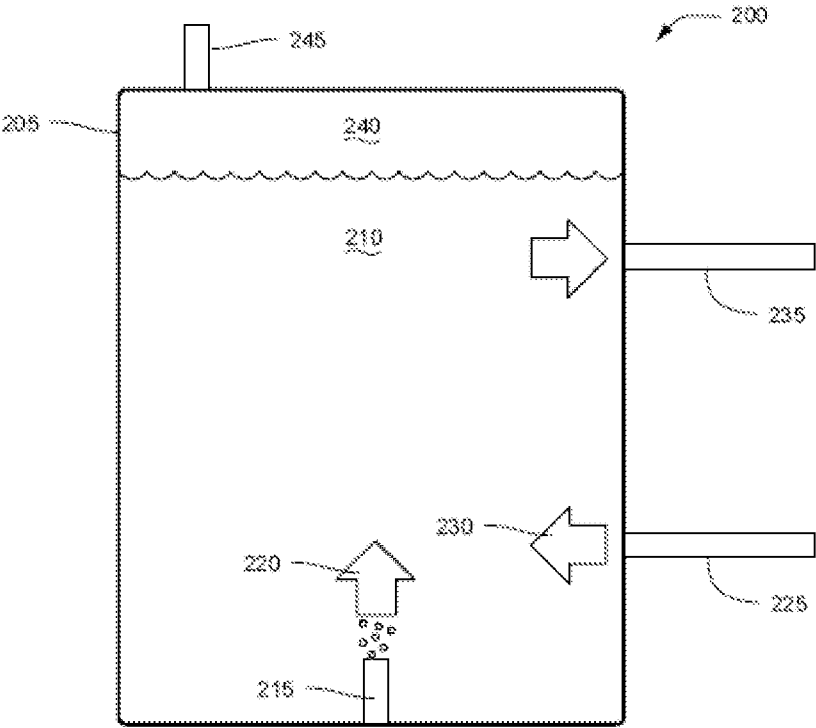


Figure 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/40557

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 1/20; C12R 1/00, 1/25 (2018.01)

CPC - C12M 43/04, 25/00, 29/18, 41/44; C12N 1/20; C12R 1/225

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013/0189763 A1 (KIVERDI, INC) 25 July 2013; paragraphs [0017], [0042]-[0043], [0045], [0048], [0077], [0113], [0149], [0194]; claims 9, 19	1-3, 4/2-3
Y	US 5266096 A (SLAVENSKY, FJ) 30 November 1993; column 2, lines 56-58; claim 1	1-3, 4/2-3
P, Y	WO 2017/165244 A1 (KIVERDI, INC) 28 September 2017; entire document	1-3, 4/2-3

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

28 August 2018 (28.08.2018)

Date of mailing of the international search report

19 SEP 2018

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/40557

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

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

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

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

3. ☒ Claims Nos.: 5-33
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.