Biofpr Biofuels Bioproducts & Biorefining

Gas fermentation of C1 feedstocks: commercialization status and future prospects

Leonardo V. Teixeira, Liza F. Moutinho, and Aline S. Romão-Dumaresq, SENAI Innovation Institute for Biosynthetics, Technology Center for Chemical and Textile Industry, Rio de Janeiro, Brazil

Received December 04, 2017; revised June 04, 2018; accepted June 05, 2018
View online at Wiley Online Library (wileyonlinelibrary.com);
DOI: 10.1002/bbb.1912; *Biofuels, Bioprod. Bioref.* (2018)

Abstract: The increasing emissions of carbon dioxide, methane and carbon oxide (collectively referred as C1 compounds) are likely to configure a major contribution to global warming and other environmental issues. The implementation of carbon capture and storage (CCS) is considered a crucial strategy to prevent global warming, but the overall costs of currently available CCS technologies are still prohibitive for its large-scale deployment. Using microorganisms capable of assimilating C1 compounds for producing value-added products could be an important driver for mitigating emissions and minimizing their deleterious consequences, while simultaneously deriving additional economic benefits from these compounds. This review summarizes the main microorganisms and metabolic routes being investigated, with special focus on both the products targeted and the current industrial initiatives. There are a number of companies investing in these routes and in some instances commercial deployment was identified. Despite the variety of commercially-appealing products, genetic manipulation of microorganisms to maximize yields and the design of technologies capable of efficiently using the gaseous feedstocks are major challenges yet to be overcome to fully unlock the potential of C1 microbiological routes. © 2018 Society of Chemical Industry and John Wiley & Sons, Ltd

Keywords: technology status; carbon dioxide; methane; biotechnology; greenhouse gas; C1 feedstock

Introduction

Many of the world's ecosystems are already overexploited and unsustainable. With the expected increase in both global population and its average per capita income, demand for natural resources will rise accordingly. In addition, climate change derived from human activities could aggravate environmental issues by affecting agricultural productivity and water supplies.[1] Despite the urgent need to restrain the increase in global average temperature emphasized in the 2015 Paris Agreement, the aggregate effect of countries' mitigation pledges in terms of annual emissions of greenhouse gases (GHG) does not seem to suffice.[2]

Based on 2016 figures, carbon dioxide (CO_2) accounts for the largest proportion of GHG emissions on a CO_2 equivalent basis, surpassing 90% of emissions, followed by methane (CH_4) (Fig. 1). In absolute values, CO_2 emissions, which were at 28.8 Gt in 2007, could reach 40.2 Gt in 2030.[5] The implementation of carbon capture and storage (CCS) is considered a crucial strategy to combat global warming, but the overall costs of currently available CCS technologies are

Correspondence to: Leonardo V. Teixeira, ISI for Biosynthetics, Technology Center for Chemical and Textile Industry, Rio de Janeiro, Brazil. E-mail: lteixeira@cetiqt.senai.br

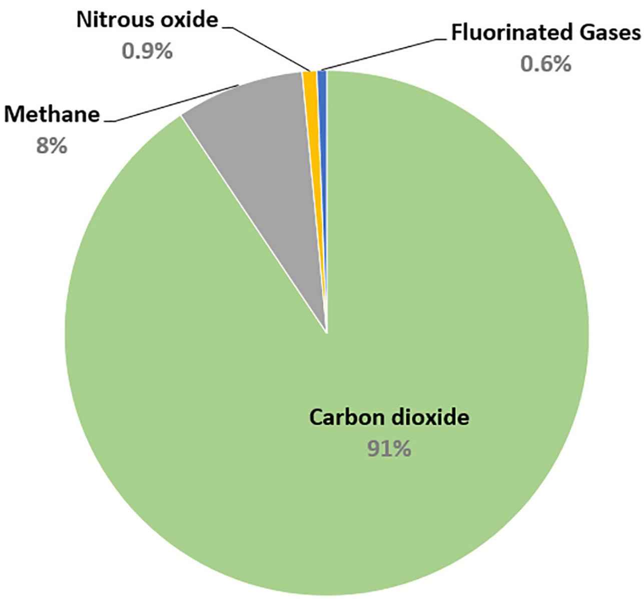

Figure 1. Global greenhouse gas (GHG) emissions breakdown on a $GtCO_2$ equivalent basis (based on 2016 data).[3] Notes: (1) CO and H_2 are excluded from the chart because they are not direct GHGs. However, they have indirect effects on global warming and climate change.[4]

still prohibitive for its large-scale deployment.[6] Using CO_2 and other one-carbon feedstocks such as CH_4 and carbon monoxide (CO) (collectively referred to as C1 feedstocks) for obtaining value-added products could be an important driver for mitigating GHG emissions and minimizing their deleterious consequences. The latter approach is known as carbon capture and utilization (CCU).

Other than the use of CH_4 as an energy source (in natural gas) and as feedstock for synthesis gas (or syngas, a mixture of CO, CO_2 and H_2), it is also employed as a precursor to chemicals, especially ammonia and methanol.[7] The uses of CO_2 include the production of urea, inorganic carbonates and pigments, methanol, salicylic acid and propylene carbonate, besides direct use in carbonated beverages and in food processing and preservation.[8,9] Finally, CO is used as a fuel, as a metallurgical reducing agent, and to produce methanol, acetic acid, phosgene, and oxo alcohols.[10] Biological production systems relying on either photosynthetic or nonphotosynthetic microorganisms that assimilate C1 feedstocks could significantly broaden this spectrum.[11] Some excellent reviews focus on the related microorganisms, metabolic routes and process advances,[11–13] but there is no current product-oriented review highlighting technologies at commercialization status. Considering the very cost-intensive and time-consuming nature of developing a microbial strain capable of feasibly producing target molecules, the knowledge of attainable products is relevant for informed decision making.

In a related matter, it would be desirable to identify metabolic pathways in which versatile metabolic intermediates are reached, as a way of unlocking different molecules of interest. Another alternative to achieve versatility is by producing the so-called platform chemicals, that is, chemical intermediates capable of yielding a large set of derivatives through physical and/or chemical transformations, targeting several distinct end uses.[14] Note that this definition is similar to that of a metabolic building block, but the former is generally transformed via chemical reactions, whereas the latter is biochemically transformed (within the microbial host).

The present paper is organized as follows. First, we describe C1 feedstocks, their sources and generation data (focusing on Brazil and the USA). Then, we provide an overview of available biochemical routes and microbial hosts using each feedstock, as well as the product applications and players involved. We finalize by outlining the most advanced initiatives, opportunities and challenges related to C1 feedstock bioconversion.

C1 Feedstocks

Many of the efforts to attain sustainable production of fuels, chemicals, and materials envision the use of biomass feedstocks.[15] First-generation sugars, obtained mainly from sugarcane and corn, are already well established and used in fermentative processes such as the production of ethanol. However, there is a concern that an expansion in fermentation processes could impose pressure on food supplies.[16] Second-generation sugars derived from lignocellulosic residues are an alternative to overcome this issue but their widespread use is still hindered by the general absence of cost-effective technologies for overcoming the residues' recalcitrance[17] and also by difficulties related to logistics and storage.[18] Besides the sugars in lignocellulosic residues, they also contain lignin ranging from 10% to 35% of the biomass[19] and, despite initiatives to add value to lignin,[20] it is mostly burned for energy generation. Alternative C1-based feedstocks for biochemical processes do not compete with food supplies or underutilize the feedstock and also have a significantly lower cost. The main ones being investigated are described below.

Syngas and CO-rich industrial Off-gases

A possible solution to use nearly all of the biomass content is its gasification to syngas, a mixture ranging from 30% to 60% CO, 25% to 30% H_2, 5% to 15% CO_2 and 0% to 5%

CH_4,[21] followed by its biochemical processing. Syngas is a traditional raw material for Fischer–Tropsch synthesis, which consists of the catalytic polymerization and hydrogenation of CO to a multiphase mixture of hydrocarbons, oxygenates, and water (syncrude). Syncrude refining yields transportation fuels and other drop-in chemicals.[22] The Fischer–Tropsch process has some drawbacks when compared with biological processes, such as high reaction temperatures (150–350 °C) and pressures (up to 30 bar), low product selectivity, greater susceptibility of the inorganic catalysts to poisoning by sulfur, chlorine and tars, and elevated costs.[23] In contrast, when performing fermentation with syngas, a relevant issue is the presence of hydrogen cyanide,[24,25] which is toxic to microorganisms.

Besides agricultural waste, municipal solid waste and organic industrial waste are potential sources of syngas. The USA alone has the potential to produce at least one billion dry tons of biomass annually without adversely affecting the environment,[26] but the current syngas production from these three feedstocks is still very limited when compared with that of coal.[27]

Besides gasification, there are other industrial sources of CO-rich streams that could be used in fermentation processes, with those from steel manufacturing receiving great attention. In Brazil, approximately 0.35 tons of carbon are emitted per ton of steel, while this number reaches 0.54 tons in the USA and 1.04 tons in China.[28] As will be described, these CO-rich streams and others present in steelmaking are already being harnessed as raw materials for bioprocesses, especially in China (by far the largest steel producer in the world).[29]

Methane (CH_4)

Another alternative C1 feedstock for biochemical processes is CH_4, the main constituent of natural gas and shale gas (>80%) and of biogas generated in anaerobic digestion processes (>50%).[30,31] Whereas nonassociated natural gas and shale gas reservoirs are abundant, concentrated sources of CH_4, natural gas associated with petroleum extraction and biogas (from agricultural and landfill waste decomposition and wastewater treatment) are small-scale, highly scattered sources. Clomburg *et* al.[12] argue that CH_4-based bioprocesses could resemble the successful example of corn-based ethanol production in the USA, which largely expanded by relying on small-scale, low-investment facilities.

In Brazil, the volume of flared natural gas surpassed 1.3 billion m^3 in 2016.[32] The country has a great potential for biogas production from agricultural and landfill waste decomposition and wastewater treatment, reaching around 52 billion m^3 per year according to recent estimates.[33] The current production of biogas is only between 0.2 and 0.6 billion m^3 annually.[34] For the sake of comparison, the current US production of CH_4 from these secondary sources is nearly 13.9 billion m^3 per year.[12]

Carbon dioxide (CO_2)

The use of CO_2 as a feedstock for fermentative processes is also being envisioned. Although being a major, concentrated source of CO_2 emissions, fossil-fuel power-plant emissions present low concentrations of CO_2 and many impurities including NO_x and SO_x compounds (especially in coal-fired units),[35] which makes CCS a more suitable option in this case. Oil refineries emit around 900 million tons of CO_2 per year, approximately the same amount as that from iron and steel production.[36]

A great deal of attention has been focused toward the very pure CO_2 generated in ethanol fermentation because it contains only minor impurities. The actual CO_2 emissions in this case are not that large. In 2008, emissions from ethanol fermentation were estimated at roughly 50 million tons worldwide (about 18.6 million tons in Brazil alone), in comparison with global fossil fuel emissions of CO_2 of 31.9 billion tons.[9] In 2017, ethanol biorefineries captured more than 2.5 million tons of CO_2 for use in food and beverage applications.[37]

Biochemical routes using C1 feedstocks

C1 feedstocks bioconversion is a rather extensive topic. Figure 2 groups the main raw materials considered for fermentation processes. Despite the great interest in microalgal photosynthetic production of biofuels, pigments, oils, nutraceuticals, and other compounds,[11] because of size limitations we have not covered them in this review.

A key aspect when it comes to C1 feedstocks is the assimilation pathway used by the microorganism. Many routes are known and Fig. 3 shows schematically those with the most commercial interest leading to pyruvate and/or acetyl-CoA, some key metabolic intermediates. Note that this is a summarized view of the metabolic routes.

Syngas- and CO-based routes

Metabolism and microorganisms

Among the microorganisms capable of metabolizing syngas (or only CO), acetogen bacteria and some archaea are the most relevant. Acetogens are anaerobic microorganisms

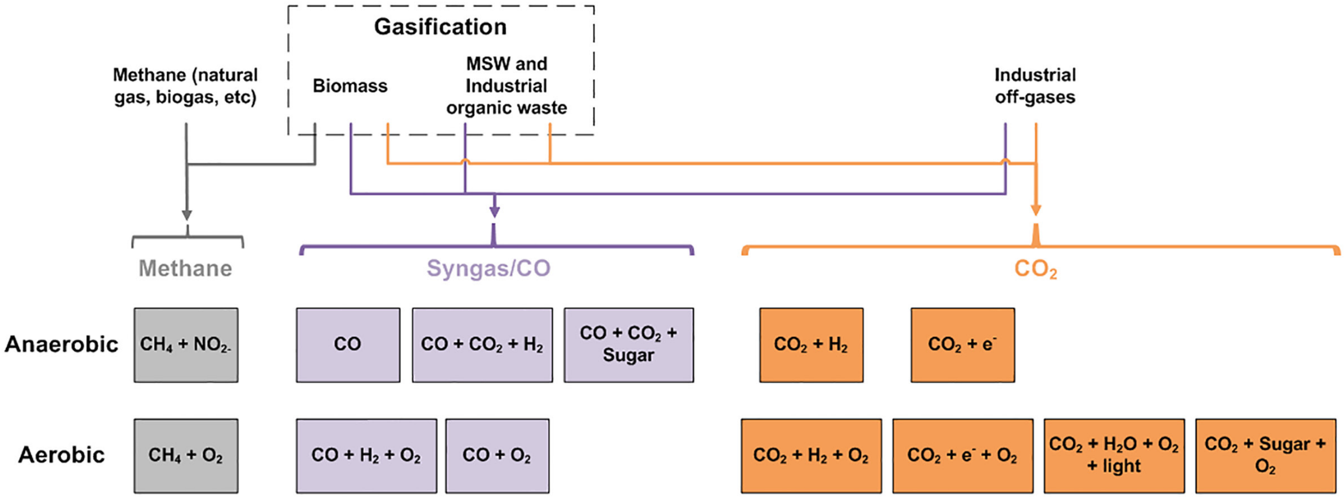

Figure 2. Summary of main C1 feedstocks used in microbiological routes (based on[13]). Note: (1) MSW, municipal solid waste.

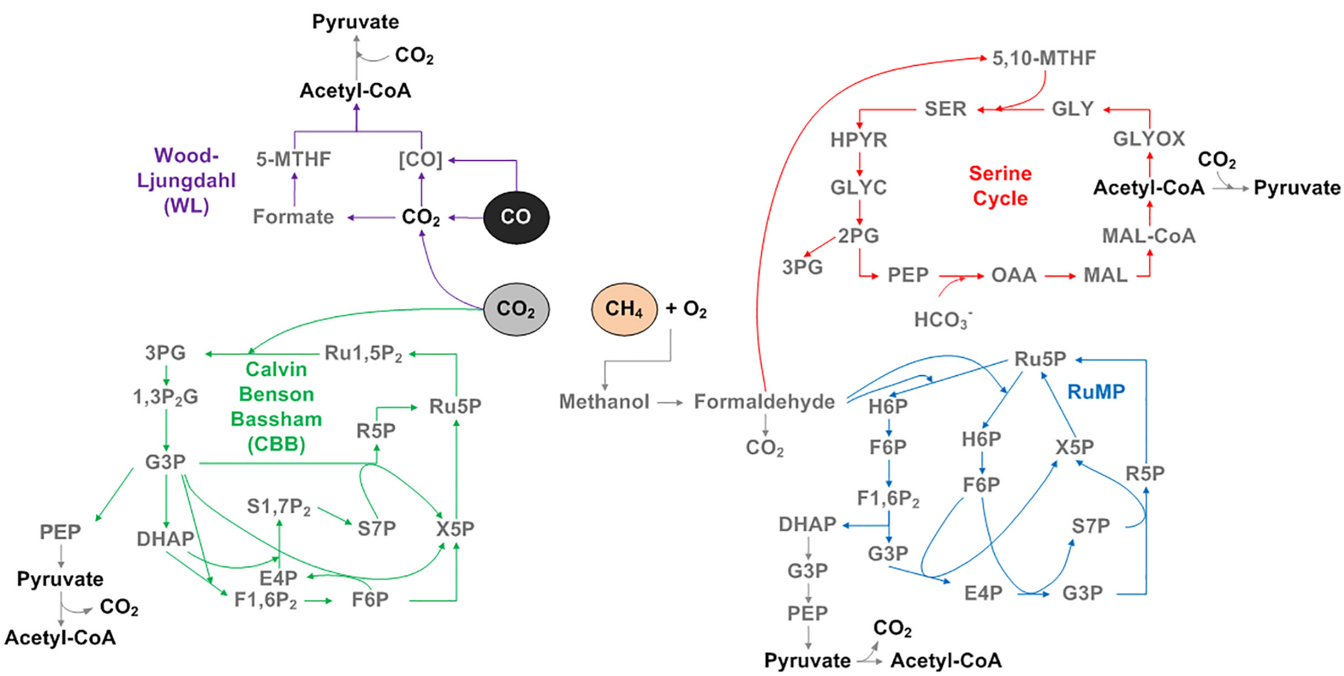

Figure 3. Selected metabolic pathways for C1 feedstock assimilation to pyruvate and/or acetyl-CoA (based on[12] and[19]). Notes: These metabolic pathways share many common intermediates and even some sequences of reactions. For the sake of clarity, they are thus presented separately. 1,3-Biphosphoglycerate, $1,3P_2G$; 2-phosphoglycerate, 2PG; 3-phosphoglycerate, 3PG; 5-methyltetrahydrofolate, 5-MTHF; 5,10-methylenetetrahydrofolate, 5,10-MTHF; dihydroxyacetone phosphate, DHAP; erythrose 4-phosphate, E4P; glyceraldehyde-3-phosphate, G3P; fructose-1,6-biphosphate, $F1,6P_2$; fructose 6-phosphate, F6P; glycine, GLY; glycerate, GLYC; glyoxylate, GLYOX; hexulose 6-phosphate, H6P; hydroxypyruvate, HPYR; malate, MAL; malonyl-CoA, MAL-CoA; oxaloacetate, OAA; phosphoenolpyruvate, PEP; ribose-5-phosphate, R5P; ribulose-1,5-phosphate, $Ru1,5P_2$; ribulose-5-phosphate, Ru5P; sedoheptulose 1,7-biphosphate, $S1,7P_2$; sedoheptulose 7-phosphate, S7P; serine, SER; xylose 5-phosphate, X5P.

ubiquitous in soils, sediments, sludge and the intestinal tracts of many animals.[38] Acetogens assimilate syngas via the linear Wood–Ljungdahl (WL) pathway (also known as the reductive acetyl-CoA pathway), in which CO uptake occurs in two possible ways (Fig. 4). Carbon monoxide can enter directly into the so-called carbonyl branch and be converted to acetyl-CoA. If additional energy is necessary, CO can be oxidized to CO_2, which enters the methyl

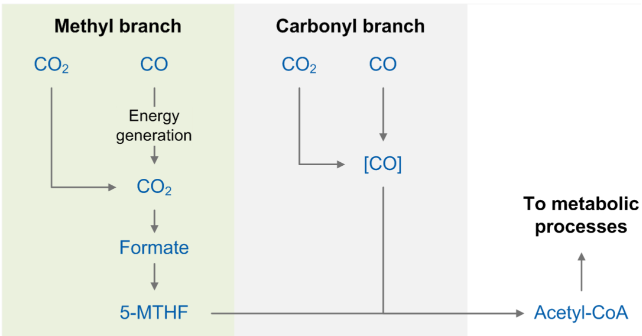

Figure 4. Simplified schematic of CO_2 and CO assimilation in the Wood–Ljungdahl pathway (based on[11]).

branch and forms formate. This energy can also be provided from H_2, but CO oxidation to CO_2 is thermodynamically more favorable. Hence, H_2 uptake is minimized in the presence of CO.[39] The fact that CO works as both energy and carbon source makes it comparatively easier to work with in relation to CO_2.[23]

The WL pathway is able to assimilate CO_2 as well. In the carbonyl branch, CO_2 is converted to CO, followed by its conversion to acetyl-CoA. In the methyl branch, CO_2 is directly converted to formate.[19]

All acetogens are able to produce acetate (acetic acid) from acetyl-CoA and specific microorganisms can derive other chemicals from this intermediate: *Clostridium ljungdahlii* (*C. ljungdahlii*), *C. autoethanogenum*, '*C. ragsdalei*' and *Alkalibaculum bacchi*, for example, are able to produce ethanol; *C. carboxidivorans* and *Butyribacterium methylotrophicum* produce *n*-butanol; *C. drakei* and *C. scatologenes* produce butyric acid; and *C. ljungdahlii*, *C. autoethanogenum* and '*C. ragsdalei*' produce 2,3-butanediol (2,3-BDO).[39]

Some of these strains naturally produce the abovementioned chemicals and part of the industrial development efforts are focused on optimizing fermentation conditions such as in ethanol production. However, the growing knowledge and expansion of genetic tools, especially for handling *C. ljungdahlii* and *C. autoethanogenum* bacteria have also allowed the development of improved recombinant strains consuming syngas/CO.[13] Another advantage of some acetogens is their mixotrophy, i.e., they can grow both autotrophically (relying on syngas/CO) and heterotrophically (consuming fructose or glucose, for example, via the glycolysis pathway).[19] The WL pathway is well suited for mixotrophy because it requires less ATP than other carbon fixation pathways and requires the exact amount of NAD(P)H generated through glycolysis to fix two molecules of CO_2 into one acetyl-CoA. Mixotrophy is not a general feature though, and the preferential consumption of sugars has been shown in *C. aceticum, for example.*[40]

Main products and current industrial initiatives

Ethanol biosynthesis is probably the most researched process of acetogens. LanzaTech, founded in 2005, is deploying two commercial ethanol-producing facilities, one in China (due by 2018)[41] and one in Belgium (due by 2019), the latter one in partnership with ArcelorMittal.[42] LanzaTech also has three commercial-scale ethanol projects under development (due by 2019)[43]: using ferroalloy off-gases (in South Africa, with Swayana),[44] refinery off gases (in India, with IndianOil),[45] and gasified orchard wood and nutshells (in California, with Aemetis).[46] The company also operates a pilot plant in Japan, employing syngas from unsorted municipal solid waste.[47]

Coskata, formed in 2006, was also addressing ethanol production in a demonstration unit, first using syngas from biomass gasification and then from reformed CH_4. Coskata went out of business in 2015 and its technology was later acquired by Synata Bio. There is no evidence of further work on such technology.[48–50] Similarly, the joint venture INEOS New Planet BioEnergy, formed in 2011, developed a syngas-to-ethanol process, but ceased operations by 2016. According to a 2014 report, a primary source of difficulty to INEOS was the high levels of hydrogen cyanide in syngas.[24,51]

LanzaTech is also investing in many molecules produced by its microbial chassis, including 2,3-BDO.[43] This molecule has a limited market, being used as a cross-linking agent for specific hard-rubber products and as an intermediate in the production of some insecticides and pharmaceuticals.[52] LanzaTech hopes to use 2,3-BDO to produce 1,3-butadiene chemically, for example; this is widely used to produce synthetic rubber.[53] Along with ethanol, the production of 2,3-BDO seems reasonably advanced.[54] In partnership with the company Invista, LanzaTech is in the early stages of development to coproduce 1,3-BDO with 2,3-BDO (instead of 2,3-BDO and ethanol). 1,3-BDO is also an intermediate to butadiene.[55] Since 2011, LanzaTech has also been working with Global Bioenergies to produce isobutylene, which is mostly used in fuels,[56] and in late 2013 it signed a three-year research cooperation agreement with Evonik to develop a technology for syngas-based specialty plastics.[57] Evonik previously demonstrated 2-hydroxyisobutyric acid (2-HIBA) production from syngas and the partnership would extend the development. 2-HIBA is an intermediate to poly(methyl methacrylate), used in transparent sheets and molded profiles.[58]

Another firm investing in syngas-based routes is the start-up White Dog Labs, founded in 2012. This firm is focused on mixotrophic fermentation (at pilot scale) using sugars and syngas/CO to produce mainly acetone and isopropanol, although it also envisions a larger portfolio of products derived from acetyl-CoA.[59,60] Acetone is widely employed as a solvent and as an intermediate to methyl methacrylate and bisphenol A, both of which are used in polymers' manufacturing,[61] while isopropanol is mainly a solvent in inks and surfactants.[62] Besides White Dog Labs, LanzaTech has also demonstrated the production of acetone and isopropanol.[63,64]

As the main product of acetogens, acetic acid is mostly used in the production of polymers derived from vinyl acetate (e.g., for paints and coatings) and from cellulose (e.g., for apparel and fibers), and also for the production of solvents.[65] Butyric acid, another common product, is a raw material in the production of lacquers, plastics, and perfumes,[66] while *n*-butanol is largely consumed in the production of surface coatings, as a solvent for varnishes or as a precursor to other solvents or monomers.[67]

CH_4-based routes

Metabolism and microorganisms

Methanotrophic bacteria are naturally found in samples from muds, swamps, rivers, oceans, sewage sludge, as well as in gas pipelines.[68] Most bacteria known to use CH_4 as their sole carbon and energy source (obligate methanotrophs) overcome the low reactivity of the C—H bond through oxygen-dependent enzymes called methane monooxygenases (MMOs), forming methanol. Methanol is then converted to formaldehyde and subsequently to CO_2.[12] The former enters the bacterial metabolism through the serine and/or the ribulose monophosphate (RuMP) cycles and methanotrophs are generally grouped according to such metabolic pathways. Group I methanotrophs are *Gammaproteobacteria* (formerly known as Type I and X) and use the RuMP pathway. Examples of Group I methanotrophs include the genera *Methylococcus, Methylomonas, Methylosphaera* and *Methylosoma*. Conversely, Group II includes *Alphaproteobacteria* (formerly Type II), which rely on the serine cycle. Examples of Group II bacteria include the genera *Methylosinus, Methylocapsa, Methylocella,* and *Methylocystis*. Carbon dioxide assimilation by methanotrophs is not common but recently discovered bacteria in the phylum Verrucomicrobia are capable of assimilating it via the Calvin Benson Bassham (CBB) cycle (reductive pentose phosphate).[31]

Oxidation of methane under anaerobic conditions is not common. Facultative anaerobic methane oxidation by 'Candidatus *Methylomirabilis oxyfera*' bacteria has been described but this microbe has not yet been successfully isolated. In this case, the nitrite ion is reduced to NO, which is then converted to N_2 and O_2. Oxygen is soon after used to form methanol by the action of MMOs.[69]

Engineering methanotrophs to produce a new chemical is technically challenging and these microbes are difficult to grow to high cell densities.[70] It is also difficult to maintain pure methanotroph cultures because many grow better in mixed cultures.[71] Using nonmethanotrophic microorganisms that have readily available tools for genetic manipulation is an alternative to allow CH_4 consumption. In a recent patent application, for example, *Komagataella pastoris* (formerly *Pichia pastoris*) is used as a host to produce malic acid.[72]

Main products and current industrial initiatives

Most research on methanotrophs is directed to the production of biopolymers (especially poly-β-hydroxybutyrate (PHB), a type of polyhydroxyalkanoate (PHA)), single-cell protein (SCP), vitamins and antibiotics,[13,31] but there are notable examples of companies investigating other relevant chemicals. The start-up NewLight Technologies (founded in 2003) is producing PHAs from CH_4 derived from biogas. The firm claimed it had successfully commissioned a commercial facility with 25 kta capacity,[73,74] but it is not clear which type of PHA it produces. A recent patent highlights the production of PHB, though.[75] Poly-β-hydroxybutyrate is mainly used in medical applications (e.g., internal sutures) because of its biocompatibility and nontoxic nature. It can also be blended to make foams, blown films, fibers and injection molding parts.[76] Mango Materials, founded in 2010, produces PHB at a pilot scale.[77]

Meanwhile, SCP are dried cells of microorganisms rich in proteins, vitamins, essential amino acids and lipids, which are used in human food or animal feed.[78] Calysta, a start-up founded in 2011, is now producing SCP from CH_4 as a fish feed ingredient and claims to be producing on a 10 kta scale.[79,80] The technology dates back to the 1980s from the efforts of the Norwegian company Norferm and it had at least two important drivers: the availability of cheap CH_4 from the North Sea and the possibility of providing SCP to companies in Norway.[81,82] Founded in 2001, the Danish firm Unibio also produces SCP from CH_4 but for animal feed and at a pilot scale.[83]

In another effort, Calysta partnered in 2013 with the lactic acid producer NatureWorks to advance a CH_4-based route to lactic acid, aiming to reduce the costs of its polylactic acid (PLA) now derived from sugars.[84] Polylactic acid is suited for packaging materials, insulation foam, automotive parts, and fibers (textile and nonwoven).[76] Another relevant product investigated by Calysta is propylene (the key monomer in the production of polypropylene), produced by *Methylosinus trichosporium* and *Methylococcus capsulatus*.[85] Calysta also looked into biofuels production because the extensive lipidic membrane of methanotrophs can be a source of hydrocarbons for biofuels.[86]

Although most companies investigate sugar-based isobutanol,[76] Intrexon demonstrated both isobutanol and farnesene production in methanotrophs. The firm, founded in 1998, currently focuses on isobutanol (pilot-scale production), but farnesene figures as a remarkable platform chemical.[87,88] Isobutanol is envisioned as a renewable fuel blendstock with a superior energy density when compared with ethanol, allowing its mixture with gasoline at higher proportions.[89] It is also a precursor to para-xylene, employed in poly(ethylene terephthalate) (PET) production, and to isobutylene. Farnesene, in turn, is mostly associated with the start-up company Amyris, which uses it to produce cosmetics, plastics, lubricants, and fuels.[90] Intrexon have also patented the biochemical production of biodiesel (fatty acid methyl esters), 2,3-BDO, *n*-butanol and fatty alcohols in *Methylococcus capsulatus*.[91] Fatty alcohols are used in surfactants for cosmetics and food products, and in lubricants.[92]

Founded in 2014, Industrial Microbes investigates the production of malic acid, a compound used in food and beverages. In recent years, the company has received successive grants to advance its technology.[93–95]

Besides syngas/CO, LanzaTech is also involved in CH_4 fermentation to chemicals,[96] but specific targets were not found. Although not using a biochemical process *per se*, an interesting approach developed by the start-up Siluria Technologies consists of using viruses as templates for nanowire catalysts, which are able to convert CH_4 to ethylene.[97] In 2014, Siluria built a demonstration plant on a Braskem US site.[98]

CO_2-based Routes

Metabolism and microorganisms

As CO_2 is the most oxidized C1 feedstock, it requires some form of energy input to produce more reduced compounds. This input can be provided in the form of light (as in photosynthetic microorganisms) or through more efficient sources of reducing power, including bioelectrocatalysis or H_2 (Fig. 2) H_2 can be derived from traditional sources, such as natural gas steam reforming, or from water electrolysis, for example. A recent patent application assigned to LanzaTech[99] proposed the latter, supplementing CO_2-rich streams from steelmaking.[1,11]

There are six known metabolic pathways for CO_2 fixation: the already discussed (1) CBB cycle and (2) the WL pathway; (3) the reductive citric acid cycle; (4) the dicarboxylate/4-hydroxybutyrate cycle; (5) the 3-hydroxypropionate/4-hydroxybutyrate cycle; and (6) the 3-hydroxypropionate bi-cycle.[12] With the exception of the latter and the WL pathway, the other four pathways are similar to one another, as they incorporate inorganic carbon into available carbon backbones, utilize acetyl-CoA/succinyl-CoA cycles, and partially overlap.[100]

Carbon dioxide fixation under both anaerobic and aerobic conditions has been demonstrated and many microorganisms possess the pathways listed. Using light as energy input, aerobic cyanobacteria have received increased attention owing to the availability of genetic tools for their manipulation (especially for *Synechocystis* and *Synechococcus*).[13] In an intriguing approach, researchers used engineered *Synechococcus elongates* to export CO_2 as sucrose, which was consumed by the PHB-producing bacterium *Halomonas boliviensis*. This consortium achieved good productivities with the additional advantage of showing enhanced resistance to microbial contaminants.[101]

In turn, H_2 can be used by the already described acetogenic bacteria to provide energy. For example, Straub *et* al.[102] showed increased acetate production in *Acetobacterium woodii* through overexpression of genes associated with the WL pathway methyl branch, which favored CO_2 assimilation. The facultative chemolithoautotrophic bacterium *Cupriavidus necator* (formerly *Ralstonia eutropha*) is also very appealing because of its ability to produce PHB assimilating CO_2 via the CBB cycle using H_2 as the sole energy source and the availability of genetic tools for its manipulation.[8] This bacterium has been employed for decades to produce PHB from sugars. Given the growing environmental concerns, attention has been driven toward the use of its autotrophic metabolism,[103] but fixation of CO_2 under heterotrophic conditions was also shown.[104] The hyperthermophilic archaeon *Pyrococcus furiosus has recently been engineered with the* 3-hydroxypropionate/4-hydroxybutyrate cycle of *Metallosphaera sedula, a* thermoacidophilic archaeon. Through the use of the available genetic tools, *P. furiosus* was shown to produce 3-hydroxypropionic acid (3-HP) from CO_2 and H_2.[105]

Bioelectrocatalysis (also referred to as microbial electrosynthesis (MES)[106]) is another interesting alternative. Microorganisms can be in suspension, transferring electrons via some chemical that functions as an electron shuttle, or can form a biofilm in the electrode, with the latter concept being more often explored.[107] Examples of microorganisms employed include the anaerobic acetogens *Sporomusa ovata* (*S. ovata*), *S. silvacetica*, *S. sphaeroides*, *C. ljungdahlii*, *C. aceticum*, and *Moorella thermoacetica*, which produced acetic acid and 2-oxobutyrate in varying quantities.[108,109] Aerobic MES tends to be inefficient compared with anaerobic MES because of the consumption of electrons for oxygen reduction.[13]

The US ARPA-E (Advanced Research Projects Agency-Energy) recently supported projects that were based on electrochemical conversion of CO_2 to formate. The greater solubility of formate tends to facilitate microbial assimilation and its biochemical conversion back to CO_2 generates energy for the microorganism.[110] Microbial electrosynthesis of isobutanol and 3-methyl-1-butanol (isoamyl alcohol) was shown in *C. necator*,[110] and isooctane MES was shown in *Escherichia coli*.[111]

Main products and current industrial initiatives

Founded in 2007, the start-up Joule Unlimited was a prominent player in photobiocatalytic CO_2 conversion using genetically modified *Synechococcus* cyanobacteria, until it went out of business in 2017 because of the inability to produce biofuels competitively in a scenario of low oil prices. It employed channeled closed photobioreactors in continuous campaigns (from 8 to 12 weeks) to produce ethanol and diesel components (e.g., *n*-alkanes) at a pilot scale.[112,113] In general, pathways relying on photosynthesis are usually constrained by drawbacks in the most common cultivation systems (open ponds and photobioreactors).[114]

Prior to its acquisition by Cargill in 2015, OPX Biotechnologies envisioned production of 3-HP and biodiesel from CO_2 (or CO) and H_2 from syngas in chemolithotrophic bacteria (e.g., *C. necator*).[115–117] 3-HP is perhaps one of the most interesting chemicals produced by anaerobes consuming CO_2. It is considered one of the main platform chemicals and can serve as an intermediate to acrylic acid (used in coatings, adhesives, diapers, paints, etc.), acrylonitrile (used in synthetic rubbers), 1,3-propanediol (used in polymers, for example) and others.[118] No information was found regarding the status of technology development, so it is assumed to be at lab scale.

In general, MES shows bottlenecks that currently hinder industrial deployment, such as compatibilization between biofilm and electrode, management of microbial growth and survival, selectivity toward target products and absence of proper methods for product recovery.[107] VITO, a research and technology organization based in Belgium, investigated MES of ethanol and ethylene. In the latter case, the first microbial consortium produced acetate (acetogenic bacteria), while the second consortium transformed acetate to ethylene (microorganisms not identified).[119,120] Financed by the US ARPA-E from 2010 to 2014, the start-up Ginkgo Bioworks investigated isooctane production based on electrochemical CO_2-to-formate conversion.[111] Propionic and butyric acids production by MES was also shown elsewhere at lab scale.[121] Propionic acid is mainly a preservative in food and feed products.[122] Microbial electrosynthesis was also used to generate isoamyl alcohol, the main component of fusel oil (a common residue in ethanol production), which is used in flavors and fragrances.[123]

There are also initiatives to produce SCP from CO_2. NovoNutrients is developing chemoautotrophic microorganisms to convert CO_2 and H_2 into feed products (SCP),[124] but the microorganisms were not identified. Kiverdi uses *C. necator* to produce SCP[125] and demonstrated pilot-scale production of fatty acids, used as surfactants or feedstock for biofuels, and hydrocarbons, used as biofuels.[126] Although LanzaTech owns a patent describing the production of SCP,[127] there is no indication of its development stage.

In comparison with other C1 feedstocks, industrial examples of CO_2 assimilation to chemicals are limited, so it is worthwhile presenting some other examples of interesting products being researched. Poly-β-hydroxybutyrate, a product described above, is a typical product of aerobic wildtype and recombinant *C. necator*.[13] The production of isoprene, a molecule extensively used to produce synthetic rubbers, has been reported using the cyanobacteria *Synechocystis*.[128]

Opportunities and challenges of the C1 biochemical platform

This section discusses the opportunities and challenges of C1-based biochemical routes, providing some market considerations on the products, insights on the technical aspects of the routes and future prospects.

The first point that deserves attention is the many endeavors to manufacture products available from sugar-based routes, some of which are already at commercial scale (e.g., ethanol, 1,3-PDO and lactic acid).[76] The potential low-cost of C1 feedstocks and the assumed superior environmental benefits are decisive aspects drawing the

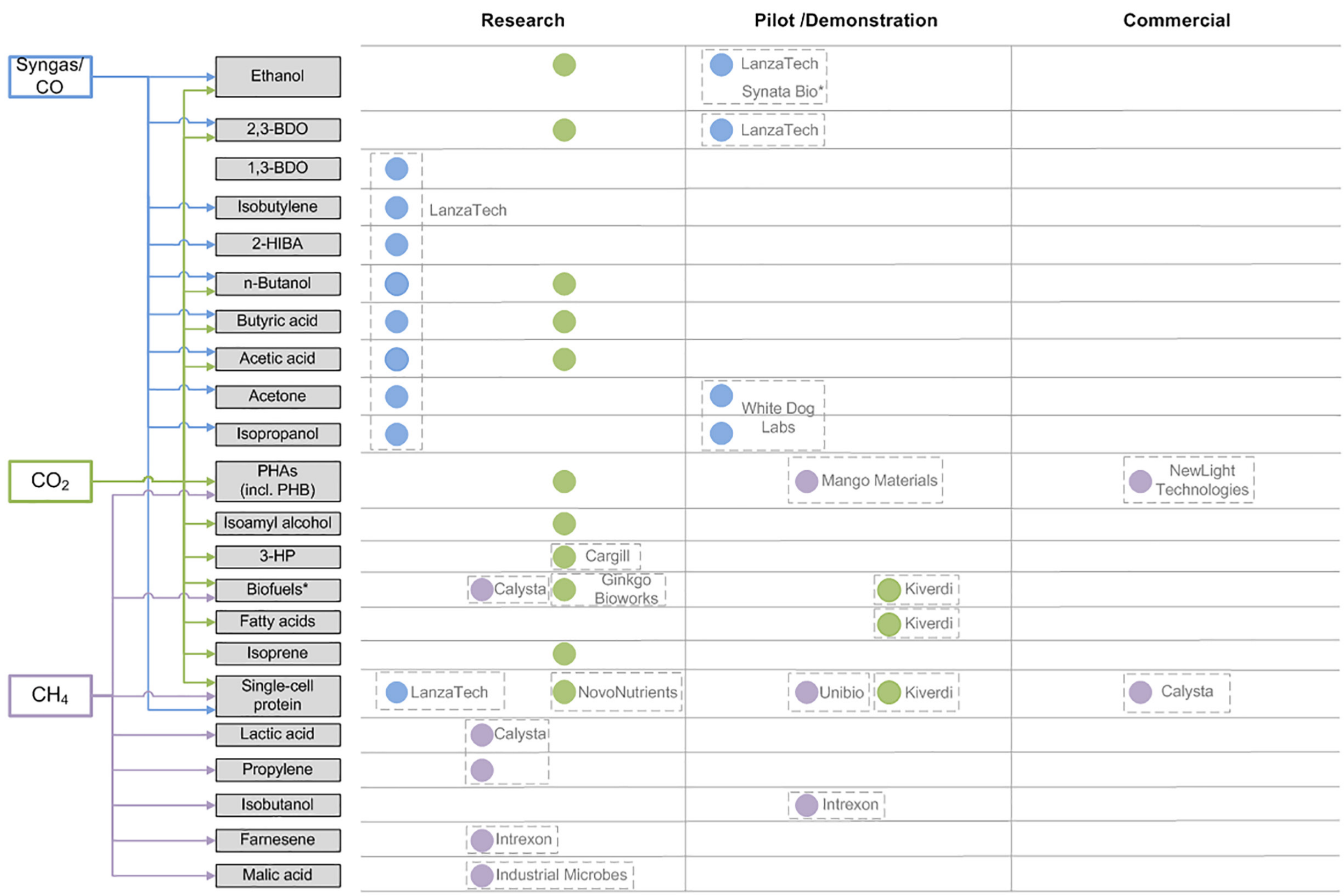

Figure 5. Development stage of the main products from C1 feedstocks. Notes: (1) The biofuels (marked with an asterisk) refer to longer chain alkanes, alkenes, and oxygenates. (2) It was not possible to find information on the current development stage of Synata Bio (marked with an asterisk) but the former Coskata ethanol technology was previously at demonstration stage.

interest of both start-ups and established companies. For products that are not as developed as ethanol from sugarcane, for example, these cheaper raw materials have the potential to make biobased products more affordable and consequently expand current markets. From a technology standpoint, the cheap feedstock also means that it is not mandatory to approach the route maximum theoretical yield to have a cost-competitive process, even if a low-value product is targeted.[129] In addition, because C1 sources are diverse and more distributed geographically, countries with limited sources of sugars or lignocellulosic residues could intensify their biobased production. This is a favorable point to firms that pursue a licensing strategy, as Coskata envisioned, for example,[130] because of the great number of potential clients.

As became clear in the previous section, there is a large number of products obtainable from C1 feedstocks, including drop-in commodities such as ethanol, acetone and propylene, non-drop-in specialties such as PHB, and platform chemicals that include 3-HP, lactic acid and farnesene.[131] Figure 5 presents the development stage of the main products as claimed by each company. We acknowledge that other parties (e.g., universities and research institutes) might also have reached at least pilot-scale production (over 100 L fermentation),[132] but we have chosen here to focus on companies. Given the comparatively smaller efforts in CO_2 routes, we also present other relevant products from this feedstock described on academic literature, but not by firms.

The list of products presented in Fig. 5 is intrinsically limited because most C1-based routes involve versatile metabolic intermediates, including acetyl-CoA and pyruvate. Acetyl-CoA occupies a central position in multiple cellular processes of many organisms, including as metabolic intermediate, precursor of anabolic reactions, allosteric regulator of enzymatic activities. It is so relevant that hypothetical reconstructions of the origin of life argue that it was involved in ancestral microbial reactions.[133] As perhaps the most versatile biological molecule, pyruvate can generate a myriad of molecules, including acetyl-CoA. Pyruvate is a key biochemical building block that participates in different catabolic and anabolic pathways. It is formed during glycolysis, the first step of cellular respiration in which glucose is broken down and can be used to build glucose through gluconeogenesis. Both acetyl-CoA and pyruvate can be substrates to yield fatty acids, aromatics, terpenoids, amino acids, organic acids, alcohols, lipids and other compounds.

Despite the potential to unlock products of interest via metabolic engineering and synthetic biology, the related tools for C1-assimilating microorganisms are not always available, as is commonplace for the sugar-consuming model organisms *E. coli* and *Saccharomyces cerevisiae*. For acetogens, there is generally a lack of both versatile genetic tools and characterization, at genetic and molecular levels, although some progress has been made for *Clostridia*. The use of *Clostridia* is also justified by the availability of tools for nonacetogenic *Clostridia* employed in ABE, acetone-butanol-ethanol (or IBE, isopropanol–butanol–ethanol) fermentation or for medical purposes. In addition, the relative simplicity of the WL pathway drives attention to acetogens, despite the complex, interconnected energy conservation mechanisms that enable microbial growth on syngas.[19,23] This set of advantages makes syngas/CO-based processes relying on acetogens a compelling alternative for the versatile production of chemicals.

In methanotrophs, the genetic tools that could be employed to divert key intermediates to desired products still need to be further developed,[12] although some basic tools were described for strains of *Methylococcus capsulatus*[134] and *Methylomicrobium buryatense*.[135] The more advanced industrial initiatives rely on natural methanotroph products, especially PHAs and SCP.

As discussed, routes relying on CO_2 suffer from the major difficulty of requiring an energy source to convert this thermodynamically stable molecule. So, the use of H_2 as an energy carrier would be advantageous given the possibility of relying on the WL pathway and, consequently, deriving products from acetyl-CoA and pyruvate. Routes using cyanobacteria such as that pursued by Joule Unlimited could also be interesting because of the presence of pyruvate in the metabolic pathways.[112] Nonetheless, further development is yet to be seen in both cases.

The challenges of engineering the various C1-consuming microorganisms impose some restrictions on the attainable products in the short term. Although interesting platform chemicals that could expand new markets are being investigated, production of drop-in commodities (especially ethanol) seems closer to large-scale commercialization. Despite the impossibility of both participating in an emerging value chain construction and acting to capture more value from the new opportunity, companies dealing with drop-in solutions have the advantage of not having to engage in market or application development.[136] Cost-competitive drop-in products from C1 feedstocks might therefore constitute an interesting business opportunity.

Other than synthetic biology, there are pending process issues to allow further development of C1-based routes. An obvious one is mass transfer from the gas to the liquid phase. For the purpose of illustration, the solubilities of CH_4, CO, CO_2, O_2 and H_2 in water at 293 K and 1 atm are approximately 24 mg L^{-1}, 28 mg L^{-1}, 1.7 g L^{-1}, 42 mg L^{-1} and 1.6 mg L^{-1}, respectively, whereas glucose solubility is 900 g L^{-1}.[137,138] Low energy-consuming bioreactor designs able to achieve homogeneous mixing of gases at high scale have been investigated.[23] Calysta, for instance, patented a loop reactor in which rapid liquid flow drives gases downward against buoyancy, causing *in situ* pressurization of the same and higher dissolution rate.[82] Unibio operates a loop reactor as well.[83,139] Based on the reactor schematics disclosed in a patent from LanzaTech,[140] its former demonstration unit located in Shanghai (China) operated with a forced loop reactor. A more recent patent though, describes a different configuration relying on bioreactors in series, which would facilitate process control. Each reactor operates with recirculation through an internal tube, driven by different pressures and densities of the liquid phase.[141]

The partial pressure of gaseous components is another important operational parameter. In the WL pathway, for example, CO and H_2 act as sources of electrons/reducing equivalents for converting CO_2 to more reduced products rather than acid products (e.g., ethanol versus acetate). Hence, their partial pressures and consequent solubility in the liquid phase drive product yield and selectivity.[23] Besides these issues, there is also a risk of explosion when employing H_2 and O_2 in fermentative processes, demanding proper countermeasures, such as keeping O_2 levels below the mixture lower detonation limit.[142]

Photosynthetic utilization of CO_2 suffers from drawbacks in the most common cultivation systems and only pilot-scale deployment has been identified. As an alternative technology, MES from CO_2 still has to surpass many technical challenges to progress beyond lab scale.[107]

To conclude, C1-based biochemical technologies still need improvements, ranging from the enhancement of microorganisms employed, to the development of robust industrial processes. There are also different entry strategies for companies investing in these technologies, including leveraging or not leveraging the versatility of the biochemical platform to diversify their products' portfolio; focusing on commodities, specialty chemicals and/or platform chemicals; owning the manufacturing facilities and/or licensing the technologies; and establishing strategic alliances (e.g., joint ventures and joint development agreements) to help develop the technologies. Nevertheless, these biochemical routes constitute an unprecedented, probably game-changing chance to reduce harmful GHG emissions and simultaneously derive economic benefits from rather inexpensive feedstocks, thus deserving the attention of researchers, industrial players and governments.

References

1. OECD, *The Bioeconomy to 2030 – Designing a Policy Agenda*. Organisation for Economic Co-operation and Development, Paris, 323 p. (2009).
2. UNFCCC, *Adoption of the Paris Agreement*. United Nations Office, Paris, 32 p. (2015).
3. Environmental Protection Agency (EPA), *GHGRP (Greenhouse Gas Reporting Program) Overview Report*. [Online]. (2016). Available: https://www.epa.gov/sites/production/files/2017-09/documents/ry16_ghgrp_overview_report.pdf [21 April 2018].
4. Intergovernmental Panel on Climate Change (IPCC), *Carbon Monoxide (CO) and Hydrogen (H2).* [Online]. Available: https://www.ipcc.ch/ipccreports/tar/wg1/139.htm [12 April 2018].
5. IEA, *World Energy Outlook*. International Energy Agency, Paris, 691 p. (2009).
6. Leung DYC, Caramanna G and Maroto-Valer MM, An overview of current status of carbon dioxide capture and storage technologies. *Renew Sustain Energy Rev* **39**:426–443 (2014).
7. Hess WT, Kurtz A and Stanton D, Methane, ethane, and propane, in *Kirk-Othmer Encyclopedia of Chemical Technology (vol. 13)*, 4th edn, ed. by Howe-Grant M. John Wiley & Sons, New York, NY, pp. 398–400 (1993).
8. Jajesniak P, Ali HEMO and Wong TS, Carbon dioxide capture and utilization using biological systems: Opportunities and challenges. *J Bioprocess Biotech* **4**(3):1–15 (2014).
9. Xu Y, Isom L and Hanna MA, Adding value to carbon dioxide from ethanol fermentations. *Bioresour Technol* **101**(10):3311–3319 (2010).
10. Kirk RE, Carbon monoxide, in *Kirk–Othmer Encyclopedia of Chemical Technology (vol. 5*), 4th edn, ed. by Howe-Grant M. Wiley-Blackwell, New York, NY, pp. 48–61 (1998).
11. Mohan SV, Modestra JA, Amulya K, Butti SK and Velvizhi G, A circular bioeconomy with biobased products from CO2 sequestration. *Trends Biotechnol* **34**(6):506–519 (2016).
12. Clomburg JM, Crumbley AM and Gonzalez R, Industrial biomanufacturing: The future of chemical production. *Science* **355**(6320):aag0804 (2017).
13. Dürre P and Eikmanns BJ. C1-carbon sources for chemical and fuel production by microbial gas fermentation. *Curr Opin Biotechnol* **35**:63–72 (2015).
14. de Araújo MR, Desenvolvimento de novas plataformas químicas: o caso do bio-ácido succínico. Master dissertation Universidade Federal do Rio de Janeiro, Rio de Janeiro (2015).
15. ABDI, *Agenda Tecnológica Setorial (ATS) – Química de Renováveis*. Agência Brasileira de Desenvolvimento Industrial, Brasília, 45 p. (2016).
16. Sims R, Taylor M, Saddler J and Mabee W, *From 1st to 2nd-Generation Biofuel Technologies – An Overview of Current Industry and RD&D Activities*. OECD/IEA, Paris, 124 p. (2008).
17. Brodeur G, Yau E, Badal K, Collier J, Ramachandran KB and Ramakrishnan S, Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. *Enzyme Res* **2011**:1–17 (2011).
18. Kim S and Dale BE, All biomass is local: The cost, volume produced, and global warming impact of cellulosic biofuels depend strongly on logistics and local conditions. *Biofuels Bioprod Biorefin* **9**(4):422–434 (2015).
19. Latif H, Zeidan AA, Nielsen AT and Zengler K, Trash to treasure: Production of biofuels and commodity chemicals via syngas fermenting microorganisms. *Curr Opin Biotechnol* **27**:79–87 (2014).
20. Biofuels Digest, *MetGen, Sweetwater Unlocking Lignin – the Roughest, Toughest, Ornieri'st Material that Ever Bushwhack'd a Pioneer in the Valley of Death*. [Online]. (2017). Available: http://www.biofuelsdigest.com/bdigest/2017/08/16/metgen-sweetwater-unlocking-lignin-the-roughest-toughest-ornierist-material-that-ever-bushwhackd-a-pioneer-in-the-valley-of-death [13 April 2018].
21. National Energy Technology Laboratory (NETL), *Syngas Composition*. [Online]. Available: https://www.netl.doe.gov/research/coal/energy-systems/gasification/gasifipedia/syngas-composition [13 April 2018].
22. de Klerk A, Fischer–Tropsch facilities at a glance, in *Fischer-Tropsch Refining*, Wiley Online Books, Weinheim. [Online]. (2011). Available: https://onlinelibrary.wiley.com/doi/10.1002/9783527635603.ch1 [13 April 2018].
23. Liew F, Martin ME, Tappel RC, Heijstra BD, Mihalcea C and Köpke M, Gas fermentation—a flexible platform for commercial scale production of low-carbon-fuels and chemicals from waste and renewable feedstocks. *Front Microbiol* **7**:694 (2016).
24. TCPalm, *INEOS Bio Selling Indian River County Biofuel Plant*. [Online]. (2016). Available: https://www.tcpalm.com/story/money/business/2016/09/02/ineos-bio-selling-indian-river-county-biofuel-plant/89823970/ [13 April 2018].
25. Broer KM, Johnston PA, Haag A and Brown RC, Resolving inconsistencies in measurements of hydrogen cyanide in syngas. *Fuel* **140**:97–101 (2015).
26. US Department of Energy (DOE), *2016 Billion-Ton Report*. [Online]. (2016). Available: https://www.energy.gov/eere/bioenergy/2016-billion-ton-report [18 April 2018].
27. National Energy Technology Laboratory (NETL), *Alternatives/Supplements to Coal – Feedstock Flexibility*. [Online]. Available: https://www.netl.doe.gov/research/coal/energy-systems/gasification/gasifipedia/feedstock [13 April 2018].
28. Intergovernmental Panel on Climate Change (IPCC), *Iron and Steel*. [Online]. Available: http://www.ipcc.ch/publications_and_data/ar4/wg3/en/ch7s7-4-1.html [13 April 2018].
29. World Steel Association, *World Steel in Figures*. [Online]. (2017). Available: https://www.worldsteel.org/en/dam/jcr:0474d208-9108-4927-ace8-4ac5445c5df8/World+Steel+in+Figures+2017.pdf [12 May 2018].

30. Conrado RJ and Gonzalez R, Envisioning the bioconversion of methane to liquid fuels. *Science* **343**(6171):621–623 (2014).
31. Fei Q, Guarnieri MT, Tao L, Laurens LML, Dowe N and Pienkos PT, Bioconversion of natural gas to liquid fuel: Opportunities and challenges. *Biotechnol Adv* **32**(3):596–614 (2014).
32. Agência Nacional do Petróleo, Gás Natural e Biocombustíveis (ANP), *Boletim Mensal da Produção de Petróleo e Gás Natural*. [Online]. (2017). Available: http://www.anp.gov.br/publicacoes/boletins-anp/2395-boletim-mensal-da-producao-de-petroleo-e-gas [3 November 2017].
33. Associação Brasileira de Biogás e Metano (ABiogás), *ABiogás|Setor no Brasil*. [Online]. (2015). Available: https://www.abiogas.org.br/setor-no-brasil [3 November 2017].
34. Mariani L, *Levantamento de unidades de produção de biogás no Brasil para fins energéticos ou Mecanismo de Desenvolvimento Limpo (MDL)*. [Online]. Presentation at: EMBRAPA, Rio de Janeiro (2015). Available: https://www.embrapa.br/documents/1355242/1529323/Biog%C3%A1sFert+3.pdf/22401089-2007-4ca2-bea5-25a932b2ea49 [30 May 2018].
35. BIO-TIC, *Industrial Biotechnology for Use of CO2 as Feedstock – Summary*. [Online]. (2014). Available: http://www.industrialbiotech-europe.eu/new/wp-content/uploads/2014/07/IB-for-Use-of-CO2-as-a-Feedstock-Summary.pdf [20 May 2018].
36. Aresta M, Dibenedetto A and Angelini A, Catalysis for the valorization of exhaust carbon: From CO2 to chemicals, materials, and fuels. Technological use of CO2. *Chem Rev* **114**(3):1709–1742 (2014).
37. RFA, *2018 Ethanol Industry Outlook*. Renewable Fuels Association, Washington, District of Columbia, 40 p. (2018).
38. Drake HL, Gößner AS and Daniel SL, Old acetogens, new light. *Ann N Y Acad Sci* **1125**(1):100–128 (2008).
39. Daniell J, Köpke M and Simpson SD, Commercial biomass syngas fermentation. *Energies* **5**(12):5372–5417 (2012).
40. Jones SW, Fast AG, Carlson ED, Wiedel CA, Au J, Antoniewicz MR *et al.*, CO2 fixation by anaerobic non-photosynthetic mixotrophy for improved carbon conversion. *Nat Commun* **7**:12800 (2016).
41. Xia C, *Beijing Iron Maker to Turn Waste Gas into Biofuels*. [Online]. (2018). Available: http://www.china.org.cn/china/2018-02/27/content_50616460.htm [10 May 2018].
42. ArcelorMittal, *Towards a Circular Economy Breakthrough: Our Partnership with LanzaTech*. [Online]. Available: http://corporate.arcelormittal.com/news-and-media/our-stories/our-partnership-with-lanzatech [10 May 2018].
43. Lanza Tech, *Facilities*. [Online]. (2018). Available: http://www.lanzatech.com/facilities/ [13 April 2018].
44. LanzaTech, *Swayana Brings Carbon Recycling to South Africa*. [Online]. (2017). Available: http://www.lanzatech.com/swayana-brings-carbon-recycling-south-africa/ [10 May 2018].
45. LanzaTech, *IndianOil and LanzaTech Sign a Statement of Intent to Construct World's First Refinery Off Gas-to-Bioethanol Production Facility in India*. [Online]. (2017). Available: http://www.lanzatech.com/indianoil-lanzatech-sign-statement-intent-construct-worlds-first-refinery-gas-bioethanol-production-facility-india/ [10 May 2018].
46. Biofuels Digest, *Commercial Time: Aemetis Embarks on $158 Million Cellulosic Ethanol Project in California*. [Online]. (2018). Available: http://www.biofuelsdigest.com/bdigest/2018/03/08/commercial-time-aemetis-embarks-on-158-million-cellulosic-ethanol-project-in-california/ [10 May 2018].
47. LanzaTech, *From Trash to Tank: Upcycling of Landfill to Fuel Demonstrated in Japan*. [Online]. (2017). Available: http://www.lanzatech.com/trash-tank-upcycling-landfill-fuel-demonstrated-japan/ [10 May 2018].
48. Biofuels Digest, *Coskata: Biofuels Digest's 2014 5-Minute Guide*. [Online]. (2014). Available: http://www.biofuelsdigest.com/bdigest/2014/03/25/coskata-biofuels-digests-2014-5-minute-guide/ [13 April 2018].
49. Merkel L, *Coskata's Thermal Bio-Syngas to Biofuels Platform*. [Online]. Presentation at: Ag Biotech Community (ABIC), Saskatoon (2010). Available: http://www.abic.ca/abic2010/html/speakers/PDF%20Web%20Presentations/Energy_Tuesday_Merkel_Loula_1100_1130.PDF [30 May 2018].
50. Biofuels Digest, *Coskata's Technology Re-emerges as Synata Bio*. [Online]. (2016). Available: http://www.biofuelsdigest.com/bdigest/2016/01/24/coskatas-technology-re-emerges-as-synata-bio/ [13 April 2018].
51. Chemical Technology, INEOS Indian River BioEnergy Center, Vero Beach, Florida. [Online]. (2017). Available: https://www.chemicals-technology.com/projects/ineosbioenergyfacili/ [13 April 2018].
52. Gräfje H, Körnig W, Weitz H-M, Reiß W, Steffan G, Diehl H *et al.*, Butanediols, butenediol, and butynediol, in *Ullmann's Encyclopedia of Industrial Chemistry*, ed. by Elvers B. Wiley-VCH, Weinhein, pp. 1–9 (2000).
53. LanzaTech, *LanzaTech Expects to Commercialize Carbon Monoxide-based Ethanol by 2013 and 2,3 Butanediol by the End of 2014*. [Online]. LanzaTech. (2012). Available: http://www.lanzatech.com/23-butanediol-bio-based-23-bdo-set-for-2014-sales/ [13 April 2018].
54. Bari DF, Syngas fermentation process. Presentation at: 32nd Annual World Petrochemical Conference, Houston, TX, [Online]. Available: https://pdfs.semanticscholar.org/presentation/c4cc/9393150499d5e0b02238e97cd61d5eba1c0b.pdf [30 May 2018]. (2017).
55. Biofuels Digest, *LanzaTech, INVISTA Find Direct Pathway to Bio-based Butadiene*. [Online]. (2015). Available: http://www.biofuelsdigest.com/bdigest/2015/12/01/lanzatech-invista-find-direct-pathway-to-bio-based-butadiene/ [13 April 2018].
56. LanzaTech, *LanzaTech and Global Bioenergies Announce New Collaboration Agreement*. [Online]. (2016). Available: http://www.lanzatech.com/lanzatech-global-bioenergies-announce-new-collaboration-agreement/ [13 April 2018].
57. Evonik, *Evonik and LanzaTech Working on Bio-processed Precursors for Specialty Plastics – Evonik Industries AG*. [Online]. (2013). Available: http://corporate.evonik.com/en/media/press_releases/pages/news-details.aspx?newsid=40447 [13 April 2018].
58. Bioplastics News, LanzaTech & Evonik alliance. [Online]. (2013). Available: https://bioplasticsnews.com/2013/12/30/lanzatech-evonik-alliance/ [13 April 2018].
59. Delaware Online, White Dog Labs looks to build Delaware's next chemical giant. [Online]. (2016). Available: https://www.delawareonline.com/story/money/small-business/2016/08/14/delawares-white-dog-labs-pioneers-new-biochemical-technology/88715126/ [13 April 2018].
60. White Dog Labs, Inc, *Start-up White Dog Labs Wins doe Grant for Technology that Could Dramatically Increase Biochemicals Yield while Decreasing Carbon Dioxide Emissions*. [Online]. (2016). Available: https://www.whitedoglabs.com/press-release-june62016/ [13 April 2018].

61. Sifiniades S and Levy A, Acetone, in *Ullmann's Encyclopedia of Industrial Chemistry*, ed. by Elvers B. Wiley-VCH, Weinhein, pp. 1–19 (2005).
62. Papa A, Propanols, in: *Ullmann's Encyclopedia of Industrial Chemistry*, ed. by Elvers B. Wiley-VCH, Weinhein, pp. 1–12 (2005).
63. LanzaTech, *Bioenergy Technologies Incubator 2: Development of a Sustainable Green Chemistry Platform for Production of Acetone and Downstream Drop-In Fuel and Commodity Products Directly from Biomass Syngas via a Novel Energy Conserving Route in Engineered Acetogenic Bacteria*. [Online]. (2017). Presentation. Available: https://www.energy.gov/sites/prod/files/2017/05/f34/Development%20of%20a%20Sustainable%20Green%20Chemistry%20Platform%20for%20Production%20of%20Acetone%20and%20downstream%20drop-in%20fuel%20and%20commodity%20products%20directly%20from%20Biomass%20Syngas.pdf [30 May 2018].
64. LanzaTech, *Pollution to Products!*. [Online]. (2017). Available: http://www.lanzatech.com/pollution-products/ [20 April 2018].
65. Cheung H, Tanke RS and Torrence GP, Acetic acid, in *Ullmann's Encyclopedia of Industrial Chemistry*, ed. by Elvers B. Wiley-VCH, Weinhein, pp. 1–30 (2005).
66. Riemenschneider W, Carboxylic acids, in *Ullmann's Encyclopedia of Industrial Chemistry*, ed. by Elvers B. Wiley-VCH, Weinhein, pp. 1–15 (2005).
67. Han H, Dämbkes G and Rupprich N, Butanols, in *Ullmann's Encyclopedia of Industrial Chemistry*, ed. by Elvers B. Wiley-VCH, Weinhein, pp. 1–12 (2005).
68. Hanson RS and Hanson TE, Methanotrophic bacteria. *Microbiol Rev* **60**(2):439–471 (1996).
69. Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MMM *et al.* Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* **464**(7288):543–548 (2010).
70. Gou Z, Xing X, Luo M, Jiang H, Han B, Wu H *et al.*, Functional expression of the particulate methane mono-oxygenase gene in recombinant Rhodococcus erythropolis. *FEMS Microbiol Lett* **263**(2):136–141 (2006).
71. Methanotroph Commons, *New to the Field?*. [Online]. (2017). Available: http://www.methanotroph.org/new-to-the-field/ [3 November 2017].
72. Helman N, Clarke E and Greenfield D, Synthetic methanotrophic and methylotrophic microorganisms. US Patent Application 20170037438 (2017).
73. Nanalyze, *Newlight Technologies and AirCarbon: Plastic from Air*. [Online]. (2016). Available: https://www.nanalyze.com/2016/02/newlight-technologies-and-aircarbon-plastic-from-air/ [3 November 2017].
74. NewLight Technologies, Inc, *Our Technology|GHG to Bioplastic*. [Online]. (2018). Available: https://www.newlight.com/technology/ [16 April 2018].
75. Herrema M, Kimmel K and Creelman E, Polyhydroxyalkanoate production and related processes. US Patent Application 20150140621A1 (2015).
76. E4tech, RE-CORD and WUR, *From the Sugar Platform to Biofuels and Biochemicals – Final Report*, London, England, 183 p. (2015).
77. Bio Based Press. *Gas Fermentation: Another Promising Biobased Technology*. [Online]. (2016). Available: https://www.biobasedpress.eu/2016/07/gas-fermentation-another-promising-biobased-technology/ [16 April 2018].
78. Nasseri AT, Rassoul-Amini S, Morowvat MH and Ghasemi Y, Single cell protein: Production and process. *Am J Food Technol* **6**(2):103–116 (2011).
79. Xconomy, Inc., *Calysta's Pivot: Another Green Energy Startup Demotes Fuel Projects*. [Online]. (2014). Available: https://www.xconomy.com/san-francisco/2014/06/26/calystas-pivot-another-green-energy-startup-demotes-fuel-projects/ [16 April 2018].
80. Calysta, *Gaseous Fermentation*. [Online]. (2017). Available: http://calysta.com/technology/gaseous-fermentation/ [16 April 2018].
81. Cameron DC, Methane as a feedstock for bio-based processes. Presentation at: ARPA-E Biological Technologies for Methane-to-Liquid Fuels Workshop, Washington, DC, [Online]. (2012). Available: https://arpa-e.energy.gov/sites/default/files/documents/files/3.%20Doug%20Cameron.pdf [30 May 2018].
82. Byrne J, *New Dawn for Gas to Feed Protein as Calysta Flags Up 2017 Production Start Date*. [Online]. (2017). Available: https://www.feednavigator.com/Article/2015/03/10/New-dawn-for-gas-to-feed-protein-as-Calysta-flags-up-2017-production-start-date [16 April 2018].
83. Unibio, *Pilot Plant*. [Online]. (2017). Available: http://www.unibio.dk/technology/pilot-plant [16 April 2018].
84. NatureWorks, *NatureWorks Launches $1 Million Lab to Research and Develop Commercial Scale Methane to Lactic Acid Fermentation*. [Online]. (2016). Available: https://www.natureworksllc.com/News-and-Events/Press-Releases/2016/03-09-16-NatureWorks-Methane-to-Lactic-Acid-Fermentation-Lab [16 April 2018].
85. Silverman JA and Purcell TJ, Propylene synthesis using engineered enzymes. US Patent Application 20150232886 (2015).
86. Calysta, *BioGTL Platform for the Conversion of Natural Gas to Fuels and Chemicals*. [Online]. (2014). Available: http://calysta.com/pdfs/AIChE_final_33114.pdf [13 April 2018].
87. Intrexon, *Engineering Microbes for Commercial Products: From Fuels and Chemicals to APIs*. [Online]. (2015). Available: https://www.dna.com/userfiles/files/Engineering%20Microbes%20for%20Commercial%20Products.pdf [13 April 2018].
88. Intrexon, *Intrexon Pilot Plant for its Methanotroph Bioconversion Platform Now Operational*. [Online]. (2016). Available: http://investors.dna.com/2016-03-30-Intrexon-Pilot-Plant-for-its-Methanotroph-Bioconversion-Platform-Now-Operational [16 April 2018].
89. Biofuels Digest, *Butamax: Biofuels Digest's 2015 5-Minute Guide*. [Online]. (2015). Available: http://www.biofuelsdigest.com/bdigest/2015/02/01/butamax-biofuels-digests-2015-5-minute-guide/ [16 April 2018].
90. Amyris, *Our Story*. [Online]. (2017). Available: https://amyris.com/our-story/ [16 April 2018].
91. Coleman WJ, Vidanes GM, Cottarel G, Muley S, Kamimura R, Javan AF *et al.*, Biological production of multi-carbon compounds from methane. US Patent 9267158 (2016).
92. Noweck K and Grafahrend W, Fatty alcohols, in *Ullmann's Encyclopedia of Industrial Chemistry*, ed. by Elvers B. Wiley-VCH, Weinhein, pp. 1–23 (2005).
93. United States Environmental Protection Agency (EPA), *U.S. EPA Awards $300,000 to East Bay Small Business that Harnesses Microbes for Green Chemistry*. [Online]. (2017). Available: https://www.epa.gov/newsreleases/us-epa-awards-

300000-east-bay-small-business-harnesses-microbes-green-chemistry [16 April 2018].

94. United States Environmental Protection Agency (EPA), *Portfolio Company Industrial Microbes Receives $100,000 from the U.S. Environmental Protection Agency (EPA)*. [Online]. (2015). Available: https://www.efund.com/single-post/2015/09/02/Portfolio-Company-Industrial-Microbes-Receives-100000-from-the-US-Environmental-Protection-Agency-EPA [16 April 2018].

95. Industrial Microbes, *Industrial Microbes Wins $500,000 Grant to Turn Greenhouse Gases into Valuable Materials*. [Online]. (2014). Available: http://www.imicrobes.com/news/2014%20 05%2019%20Industrial%20Microbes%20CCEMC.pdf [16 April 2018].

96. LanzaTech, *Gas Fermentation and Modular Manufacturing*. [Online]. (2015). Available: https://www.ameslab.gov/sites/default/files/Laurel%20Harmon%20Gas%20Fermentation%20 and%20Modular%20Manufacturing%20-%20LanzaTech.pdf [16 April 2018].

97. MIT Technology Review, *Siluria Taps Natural Gas Glut to Make Chemicals*. [Online]. (2012). Available: https://www.technologyreview.com/s/428636/siluria-taps-natural-gas-glut-to-make-chemicals/ [16 April 2018].

98. ICIS News, *Siluria to Build Methane-to-C2 Demo Plant on Braskem US Site*. [Online]. (2014). Available: https://www.icis.com/resources/news/2014/01/15/9744050/siluria-to-build-methane-to-c2-demo-plant-on-braskem-us-site/ [16 April 2018].

99. Simpson SD, Conrado RJ and Mihalcea CD, Integrated fermentation and electrolysis process. US Patent Application 20170218404 A1 (2017).

100. Ducat DC and Silver PA, Improving carbon fixation pathways. *Curr Opin Chem Biol* **16**(3):337–344 (2012).

101. Weiss TL, Young EJ and Ducat DC, A synthetic, light-driven consortium of cyanobacteria and heterotrophic bacteria enables stable polyhydroxybutyrate production. *Metab Eng* **44**:236–245 (2017).

102. Straub M, Demler M, Weuster-Botz D and Dürre P, Selective enhancement of autotrophic acetate production with genetically modified Acetobacterium woodii. *J Biotechnol* **178**:67–72 (2014).

103. Müller J, MacEachran D, Burd H, Sathitsuksanoh N, Bi C, Yeh Y-C *et al.*, Engineering of Ralstonia eutropha H16 for autotrophic and heterotrophic production of methyl ketones. *Appl Environ Microbiol* **79**(14):4433–4439 (2013).

104. Shimizu R, Chou K, Orita I, Suzuki Y, Nakamura S and Fukui T, Detection of phase-dependent transcriptomic changes and Rubisco-mediated CO2 fixation into poly (3-hydroxybutyrate) under heterotrophic condition in Ralstonia eutropha H16 based on RNA-seq and gene deletion analyses. *BMC Microbiol* **13**:169 (2013).

105. Keller MW, Schut GJ, Lipscomb GL, Menon AL, Iwuchukwu IJ, Leuko TT *et al.*, Exploiting microbial hyperthermophilicity to produce an industrial chemical, using hydrogen and carbon dioxide. *Proc Natl Acad Sci* **110**(15):5840–5845 (2013).

106. Rabaey K and Rozendal RA, Microbial electrosynthesis — revisiting the electrical route for microbial production. *Nat Rev Microbiol* **8**(10):706–716 (2010).

107. Desloover J, Arends JBA, Hennebel T and Rabaey K, Operational and technical considerations for microbial electrosynthesis. *Biochem Soc Trans* **40**(6):1233–1238 (2012).

108. Nevin KP, Hensley SA, Franks AE, Summers ZM, Ou J, Woodard TL *et al.*, Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. *Appl Environ Microbiol* **77**(9):2882–2886 (2011).

109. Nevin KP, Woodard TL, Franks AE, Summers ZM and Lovley DR, Microbial electrosynthesis: Feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. *mBio* **1**(2):e00103– e00110 (2010).

110. Li H, Opgenorth PH, Wernick DG, Rogers S, Wu T-Y, Higashide W *et al.*, Integrated electromicrobial conversion of CO2 to higher alcohols. *Science* **335**(6070):1596 (2012).

111. ARPA-E, *Biofuels from E. coli*. [Online]. (2010). Available: https://arpa-e.energy.gov/?q=slick-sheet-project/biofuels-e-coli [16 April 2018].

112. Joule Unlimited, *Photobiocatalytic Conversion of CO2 to Drop-in Fuels: A Primer*. Joule, Bedford, MA, 16 p. (2015).

113. Biofuels Digest, *Heat Death: Joule Unlimited Collapses as Oil Prices Flag, Time Passes, Pressure Mounts*. [Online]. (2017). Available: http://www.biofuelsdigest.com/bdigest/2017/07/18/heat-death-joule-unlimited-collapses-as-oil-prices-fall-time-passes-pressure-mounts/ [16 April 2018].

114. Narala RR, Garg S, Sharma KK, Thomas-Hall SR, Deme M, Li Y *et al.*, Comparison of microalgae cultivation in photobioreactor, open raceway pond, and a two-stage hybrid system. *Front Energy Res* **4**:29. (2016).

115. Lynch MD, Methods, systems, and compositions for microbial bio-production of biomolecules using syngas components, or sugars, as feedstocks. US Patent Application 20110124063 (2011).

116. Cargill, *Cargill Acquires OPX Biotechnologies' Fermentation Technology*. [Online]. (2015). Available: https://www.cargill.com/news/releases/2015/NA31794255.jsp [16 April 2018].

117. Hogsett DA, OPX Biotechnologies Inc. – The leading EDGE® bio-based chemicals business. Presentation at: Advanced Biofuels Leadership Conference, Washington, DC (2014). Available: http://www.ascension-publishing.com/ABLC-2014/Hogsett-OPX.pdf [30 May 2018].

118. NREL, *Top Value Added Chemicals from Biomass*, *Volume I – Results of Screening for Potential Candidates from Sugars and Synthesis Gas* National Renewable Energy Laboratory, Golden, CO, 67 p. (2004).

119. VITO, *Electro Conversion to Chemicals*. [Online]. (2017). Available: https://vito.be/en/chemistry/efficient-reactions/electro-conversion-to-chemicals [16 April 2018].

120. European Comission: Community Research and Development Information Center (CORDIS). *Projects and Results : Final Report Summary – BIO-ELECTRO-ETHYLENE (Integrated Bio-Electrochemical Production of Ethylene through CO2 Sequestration)*. [Online]. (2016). Available: https://cordis.europa.eu/result/rcn/191430_en.html [16 April 2018].

121. Modestra JA, Navaneeth B and Mohan SV, Bio-electrocatalytic reduction of CO2: Enrichment of homoacetogens and pH optimization towards enhancement of carboxylic acids biosynthesis. *J CO2 Util* **10**:78–87 (2015).

122. Samel U, Kohler W, Gamer AO and Keuser U, Propionic acid and derivatives, in *Ullmann's Encyclopedia of Industrial Chemistry*, ed. by Elvers B. Wiley-VCH, Weinhein, pp. 1–18 (2005).

123. Burdock GA, *Fenaroli's Handbook of Flavor Ingredients*, 6th edn, CRC Press, Boca Raton, FL (2010).

124. Sefton B, Our approach: Biological CO2 capture and conversion using hydrogen-powered bacteria. Presentation at: NovoNutrients, Sunnyvale, CA. [Online]. Available: http://

nas-sites.org/dels/files/2018/02/2-2-SEFTON-NovoNutrients-NAS.pdf [18 April 2018].
125. Reed JS, Geller J and Hande S, Microorganisms and artificial ecosystems for the production of protein, food, and useful co-products from C1 substrates. WO Patent Application 2017165244 (2017).
126. Kiverdi, Inc., *CO2 Conversion by Knallgas Microorganisms: Evaluation of Products and Processes*. US Energy Research and Development Division, Berkeley, CA, 117 p. (2017).
127. Simpson S, Allen WE, Conrado RJ and Molloy S, Gas fermentation for the production of protein or feed. US Patent Application 20160338380A1 (2016).
128. Lindberg P, Park S and Melis A, Engineering a platform for photosynthetic isoprene production in cyanobacteria, using Synechocystis as the model organism. *Metab Eng* **12**(1):70–79 (2010).
129. Madrona Y, *Scientists Seek to Engineer Microbes to Make Simple Chemicals*. [Online]. (2015). Available: https://synapse.ucsf.edu/articles/2015/01/09/scientists-seek-engineer-microbes-make-simple-chemicals [16 April 2018].
130. Corley R, Coskata's Syngas to biofuels & chemicals platform. Presentation at: Gasification Technologies Conference, Vancouver. [Online]. (2016). Available: https://www.global-syngas.org/uploads/eventLibrary/16CORLEY.pdf [25 May 2018].
131. Bozell JJ and Petersen GR, Technology development for the production of biobased products from biorefinery carbohydrates—the US Department of Energy's "Top 10" revisited. *Green Chem* **12**(4):539–554 (2010).
132. Kundiyana DK, Huhnke RL and Wilkins MR, Syngas fermentation in a 100-L pilot scale fermentor: Design and process considerations. *J Biosci Bioeng* **109**(5):492–498 (2010).
133. Pietrocola F, Galluzzi L, Bravo-San Pedro JM, Madeo F and Kroemer G, Acetyl coenzyme A: A central metabolite and second messenger. *Cell Metab* **21**(6):805–821 (2015).
134. Welander PV and Summons RE, Discovery, taxonomic distribution, and phenotypic characterization of a gene required for 3-methylhopanoid production. *Proc Natl Acad Sci* **109**(32):12905–12910 (2012).
135. Puri AW, Owen S, Chu F, Chavkin T, Beck DAC, Kalyuzhnaya MG *et al.*, Genetic tools for the industrially promising methanotroph methylomicrobium buryatense. *Appl Environ Microbiol* **81**(5):1775–1781 (2015).
136. Teixeira LV, Estratégias em Indústrias Emergentes: Um Estudo de Empresas Petroquímicas e Startups na Bioeconomia. Master dissertation Universidade Federal do Rio de Janeiro, Rio de Janeiro (2016).
137. Wikipedia, *Glucose*. [Online]. (2018). Available: https://en.wikipedia.org/w/index.php?title=Glucose&oldid=832369685 [16 April 2018].
138. The Engineering ToolBox. *Solubility of Gases in Water*. [Online]. (2017). Available: https://www.engineeringtoolbox.com/gases-solubility-water-d_1148.html [16 April 2018].
139. Larsen EB, U-shape and/or nozzle u-loop fermentor and method of carrying out a fermentation process. US Patent 6492135 (2002).
140. Li X, Cossey BJ and Trevethick SR, Fermentation of gaseous substrates. US Patent 9617509 (2017).
141. Trevethick SR, Bromley JC, Waters GW, Koepke M, Tran LP and Jensen Overgaard R, Multi-Stage Biorreactor Processes. US Patent Application 20160115505 (2016).
142. Ishizaki A, Tanaka K, Takeshita T, Kanemaru T, Shimoji T and Kawano T, Equipment and operation for fermentative PHB production using gaseous substrate to guarantee safety from explosion. *J Chem Eng Jpn* **26**(2):225–227 (1993).

Leonardo V. Teixeira

Leonardo V. Teixeira is a researcher at the SENAI Innovation Institute for Biosynthetics (ISI Biossintéticos), Brazil, and a PhD student at the Federal University of Rio de Janeiro (UFRJ), Brazil. His research focuses on technology and innovation management in the bio-based industry.

Liza F. Moutinho

Liza F. Moutinho is a researcher at the SENAI Innovation Institute for Biosynthetics, Brazil, and recently graduated with a BSc in bioprocesses engineering at the Federal University of Rio de Janeiro. Her research focuses on the development of biotechnological platforms aiming to add value to renewable feedstocks.

Aline S. Romão-Dumaresq

Aline S. Romão-Dumaresq is the head researcher in biotechnology and synthetic biology at the SENAI Innovation Institute for Biosynthetics, Brazil. She works on several projects in these fields, developing solutions for industrial clients.