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, Bioproducts & Biorefining (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. 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. 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. 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
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. 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. 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.

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 and also by difficulties related to logistics and storage. Besides the sugars in lignocellulosic residues, they also contain lignin ranging from 10% to 35% of the biomass and, despite initiatives to add value to lignin, 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% H₂, 5% to 15% CO₂ and 0% to 5%
In contrast, when performing fermentation because of size x 36 and many 37 -emissions, fossil-fuel power-plant from these 4 -based bioprocesses x 2 , natural gas associated with petroleum extraction and 32 -generated in ethanol fermentation because 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 (CH₄)**

Another alternative C₁ feedstock for biochemical processes is CH₄, 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 CH₄, 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 CH₄-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 m³ 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 m³ per year according to recent estimates. 33 The current production of biogas is only between 0.2 and 0.6 billion m³ annually. 34 For the sake of comparison, the current US production of CH₄ from these secondary sources is nearly 13.9 billion m³ per year. 12

**Carbon dioxide (CO₂)**

The use of CO₂ as a feedstock for fermentative processes is also being envisioned. Although being a major, concentrated source of CO₂ emissions, fossil-fuel power-plant emissions present low concentrations of CO₂ and many impurities including NOₓ and SO₂ 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 CO₂ 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 CO₂ generated in ethanol fermentation because it contains only minor impurities. The actual CO₂ 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 CO₂ of 31.9 billion tons. 8 In 2017, ethanol biorefineries captured more than 2.5 million tons of CO₂ 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 CO₂-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

CH₄, 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.
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

ubiquitous in soils, sediments, sludge and the intestinal tracts of many animals. Acetogens assimilate syngas via the linear Wood–Ljungdahl (WL) pathway (also known as the reductive acetyl-CoA pathway), in which CO uptake

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$_2$G; 2-phosphoglycerate, 2PG; 3-phosphoglycerate, 3PG; 5-methyltetrahydrofolate, 5-MTHF; 5,10-methylenetetrahydrofolate, 5,10-MTHF; dihydroxyacetone phosphate, DHAP; erythrose 4-phosphate, E4P; 2-phosphoglycerate-3-phosphate, G3P; fructose-1,6-biphosphate, F1,6P$_2$; fructose 6-phosphate, F6P; glycine, GLY; glycerate, GLYC; glyoxylate, GLYX; hexulose 6-phosphate, H6P; hydroxypropionate, 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; xylulose 5-phosphate, X5P.
branch and forms formate. This energy can also be provided from H₂, but CO oxidation to CO₂ is thermodynamically more favorable. Hence, H₂ uptake is minimized in the presence of CO.⁵⁹ The fact that CO works as both energy and carbon source makes it comparatively easier to work with in relation to CO₂.²³

The WL pathway is able to assimilate CO₂ as well. In the carbonyl branch, CO₂ is converted to CO, followed by its conversion to acetyl-CoA. In the methyl branch, CO₂ is directly converted to formate.⁹

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. scatogenes 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 CO₂ 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.⁵² LanzaTech also has three commercial-scale ethanol projects under development (due by 2019)⁴²: 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).⁶ 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 CH₄. 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.⁴⁶⁻⁵⁰ 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 from biomass gasification and then from reformed CH₄. There is no evidence of further work on such technology.⁴⁶⁻⁵⁰ 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 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.²⁴ In partnership with the company Invisa, 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.²⁵ 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 startup 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.\(^\text{59,60}\) 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.\(^\text{61}\) While isopropanol is mainly a solvent in inks and surfactants.\(^\text{62}\) Besides White Dog Labs, LanzaTech has also demonstrated the production of acetone and isopropanol.\(^\text{63,64}\)

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.\(^\text{65}\) Butyric acid, another common product, is a raw material in the production of lacquers, plastics, and perfumes,\(^\text{66}\) 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.\(^\text{67}\)

### 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.\(^\text{58}\) 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\(_2\).\(^\text{12}\) 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 \textit{Gammaproteobacteria} (formerly known as Type I and X) and use the RuMP pathway. Examples of Group I methanotrophs include the genera \textit{Methylcoccus}, \textit{Methylomonas}, \textit{Methylisphaera} and \textit{Methylosoma}. Conversely, Group II includes \textit{Alphaproteobacteria} (formerly Type II), which rely on the serine cycle. Examples of Group II bacteria include the genera \textit{Methylosinus}, \textit{Methylcapsa}, \textit{Methylcello} and \textit{Methylocystis}. Carbon dioxide assimilation by methanotrophs is not common but recently discovered bacteria in the phylum \textit{Verrucomicrobia} are capable of assimilating it via the Calvin Benson Bassham (CBB) cycle (reductive pentose phosphate).\(^\text{31}\)

Oxidation of methane under anaerobic conditions is not common. Facultative anaerobic methane oxidation by \textit{‘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\(_2\), which is then converted to N\(_2\) and O\(_2\). Oxygen is soon after used to form methanol by the action of MMOs.\(^\text{69}\)

Engineering methanotrophs to produce a new chemical is technically challenging and these microbes are difficult to grow to high cell densities.\(^\text{70}\) It is also difficult to maintain pure methanotroph cultures because many grow better in mixed cultures.\(^\text{71}\) 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, \textit{Komagataella pastoris} (formerly \textit{Pichia pastoris}) is used as a host to produce malic acid.\(^\text{72}\)

### Main products and current industrial initiatives

Most research on methanotrophs is directed to the production of biopolymers (especially poly-\(\beta\)-hydroxybutyrate (PHB), a type of polyhydroxyalkanoate (PHA)), single-cell protein (SCP), vitamins and antibiotics,\(^\text{13,31}\) 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,\(^\text{73,74}\) but it is not clear which type of PHA it produces. A recent patent highlights the production of PHB, though.\(^\text{75}\) Poly-\(\beta\)-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.\(^\text{76}\) Mango Materials, founded in 2010, produces PHB at a pilot scale.\(^\text{77}\)

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.\(^\text{78}\) 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.\(^\text{79,80}\) 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.\(^\text{81,82}\) Founded in 2001, the Danish firm Unibio also produces SCP from CH\(_4\) but for animal feed and at a pilot scale.\(^\text{83}\)

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 poly-lactic acid (PLA) now derived from sugars.\(^\text{84}\) Polylactic
acid is suited for packaging materials, insulation foam, automotive parts, and fibers (textile and nonwoven). Another relevant product investigated by Calysta is propylene (the key monomer in the production of polypropylene), produced by *Methylosinus trichosporium* and *Methylococcus capsulatus*.

Intrexon also looked into biofuels production because the extensive lipidic membrane of methanotrophs can be a source of hydrocarbons for biofuels. Although most companies investigate sugar-based isobutanol, 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. 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. 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. Intrexon have also patented the biochemical production of biodiesel (fatty acid methyl esters), 2,3-butanediol, n-butanol and fatty alcohols in *Methylococcus capsulatus*. Fatty alcohols are used in surfactants for cosmetics and food products, and in lubricants.

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.

Besides syngas/CO, LanzaTech is also involved in CH₄ fermentation to chemicals, 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 CH₄ to ethylene. In 2014, Siluria built a demonstration plant on a Braskem US site.

### CO₂-based Routes

**Metabolism and microorganisms**

As CO₂ 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 H₂ (Fig. 2) H₂ 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 proposed the latter, supplementing CO₂-rich streams from steelmaking.

There are six known metabolic pathways for CO₂ 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. 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.

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*). In an intriguing approach, researchers used engineered *Synechococcus elongates* to export CO₂ 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.

In turn, H₂ can be used by the already described acetogenic bacteria to provide energy. For example, Straub et al. showed increased acetate production in *Acetobacterium woodii* through overexpression of genes associated with the WL pathway methyl branch, which favored CO₂ assimilation. The facultative chemolithoautotrophic bacterium *Cupriavidus necator* (formerly *Ralstonia eutrophus*) is also very appealing because of its ability to produce PHB assimilating CO₂ via the CBB cycle using H₂ as the sole energy source and the availability of genetic tools for its manipulation. 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, but fixation of CO₂ under heterotrophic conditions was also shown. The hyperthermophilic archaean *Pyrococcus furiosus* has recently been engineered with the 3-hydroxypropionate/4-hydroxybutyrate cycle of *Metallosphaera sedula*, a thermoacidophilic archaean. Through the use of the available genetic tools, *P. furiosus* was shown to produce 3-hydroxypropionic acid (3-HP) from CO₂ and H₂.

Bioelectrocatalysis (also referred to as microbial electrosynthesis (MES)) 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. Examples of microorganisms employed include the anaerobic acetogens Sporomusa ovata (S. ovata), S. silvacetica, S. sphaeroides, C. ljungdahlii, C. acetium, and Moorella thermoacetica, which produced acetic acid and 2-oxobutyrate in varying quantities.\textsuperscript{108,109} Aerobic MES tends to be inefficient compared with anaerobic MES because of the consumption of electrons for oxygen reduction.\textsuperscript{13}

The US ARPA-E (Advanced Research Projects Agency-Energy) recently supported projects that were based on electrochemical conversion of CO\textsubscript{2} to formate. The greater solubility of formate tends to facilitate microbial assimilation and its biochemical conversion back to CO\textsubscript{2} generates energy for the microorganism.\textsuperscript{110} Microbial electrolysytis of isobutanol and 3-methyl-1-butanol (isoamyl alcohol) was shown in C. necator,\textsuperscript{110} and isoctane MES was shown in Escherichia coli.\textsuperscript{111}

Main products and current industrial initiatives

Founded in 2007, the start-up Joule Unlimited was a prominent player in photobiocatalytic CO\textsubscript{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.\textsuperscript{112,113} In general, pathways relying on photosynthesis are usually constrained by drawbacks in the most common cultivation systems (open ponds and photobioreactors).\textsuperscript{114}

Prior to its acquisition by Cargill in 2015, OPX Biotechnologies envisioned production of 3-HP and biodiesel from CO\textsubscript{2} (or CO) and H\textsubscript{2} from syngas in chemolithotrophic bacteria (e.g., C. necator).\textsuperscript{115–117} 3-HP is perhaps one of the most interesting chemicals produced by anaerobes consuming CO\textsubscript{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.\textsuperscript{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.\textsuperscript{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).\textsuperscript{119,120} Financed by the US ARPA-E from 2010 to 2014, the start-up Ginkgo Bioworks investigated isoctane production based on electrochemical CO\textsubscript{2}-to-formate conversion.\textsuperscript{111} Propionic and butyric acids production by MES was also shown elsewhere at lab scale.\textsuperscript{121} Propionic acid is mainly a preservative in food and feed products.\textsuperscript{122} Microbial electrolysytis 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.\textsuperscript{123}

There are also initiatives to produce SCP from CO\textsubscript{2}. NovoNutrients is developing chemoautotrophic microorganisms to convert CO\textsubscript{2} and H\textsubscript{2} into feed products (SCP),\textsuperscript{124} but the microorganisms were not identified. Kiverdi uses C. necator to produce SCP\textsuperscript{125} and demonstrated pilot-scale production of fatty acids, used as surfactants or feedstock for biofuels, and hydrocarbons, used as biofuels.\textsuperscript{126} Although LanzaTech owns a patent describing the production of SCP,\textsuperscript{127} there is no indication of its development stage.

In comparison with other C1 feedstocks, industrial examples of CO\textsubscript{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.\textsuperscript{13} The production of isoprene, a molecule extensively used to produce synthetic rubbers, has been reported using the cyanobacteria Synechocystis.\textsuperscript{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).\textsuperscript{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. 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, 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. 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), but we have chosen here to focus on companies. Given the comparatively smaller efforts in CO₂ 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.\textsuperscript{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 \textit{E. coli} and \textit{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 \textit{Clostridia}. The use of \textit{Clostridia} is also justified by the availability of tools for nonacetogenic \textit{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.\textsuperscript{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,\textsuperscript{12} although some basic tools were described for strains of \textit{Methylococcus capsulatus}\textsuperscript{134} and \textit{Methylomonas barytovense}.\textsuperscript{135} The more advanced industrial initiatives rely on natural methanotroph products, especially PHAs and SCP.

As discussed, routes relying on CO\textsubscript{2} suffer from the major difficulty of requiring an energy source to convert this thermodynamically stable molecule. So, the use of H\textsubscript{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.\textsuperscript{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 platforms 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.\textsuperscript{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\textsubscript{4}, CO, CO\textsubscript{2}, O\textsubscript{2} and H\textsubscript{2} in water at 293 K and 1 atm are approximately 24 mg L\textsuperscript{-1}, 28 mg L\textsuperscript{-1}, 1.7 g L\textsuperscript{-1}, 42 mg L\textsuperscript{-1} and 1.6 mg L\textsuperscript{-1}, respectively, whereas glucose solubility is 900 g L\textsuperscript{-1}.\textsuperscript{137,138} Low energy-consuming bioreactor designs able to achieve homogeneous mixing of gases at high scale have been investigated.\textsuperscript{23} Calysta, for instance, patented a loop reactor in which rapid liquid flow drives gases downward against buoyancy, causing \textit{in situ} pressurization of the same and higher dissolution rate.\textsuperscript{82} Unibio operates a loop reactor as well.\textsuperscript{83,139} Based on the reactor schematics disclosed in a patent from LanzaTech,\textsuperscript{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.\textsuperscript{141}

The partial pressure of gaseous components is another important operational parameter. In the WL pathway, for example, CO and H\textsubscript{2} act as sources of electrons/reducing equivalents for converting CO\textsubscript{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.\textsuperscript{23} Besides these issues, there is also a risk of explosion when employing H\textsubscript{2} and O\textsubscript{2} in fermentative processes, demanding proper countermeasures, such as keeping O\textsubscript{2} levels below the mixture lower detonation limit.\textsuperscript{142}

Photosynthetic utilization of CO\textsubscript{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\textsubscript{2} still has to surpass many technical challenges to progress beyond lab scale.\textsuperscript{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
LV Teixeira, LF Moutinho, AS Romão-Dumaresq

Review: Commercialization status of gas fermentation of C1 feedstocks


Review: Commercialization status of gas fermentation of C1 feedstocks

LV Teixeira, LF Moutinho, AS Romão-Dumaresq


Review: Commercialization status of gas fermentation of C1 feedstocks

LV Teixeira, LF Moutinho, AS Romão-Dumaresq


Review: Commercialization status of gas fermentation of C1 feedstocks

Leonardo V. Teixeira, LF Moutinho, AS Romão-Dumaresq

nas-sites.org/dels/files/2018/02/2-2-SEFTON-NovoNutrients-NAS.pdf [18 April 2018].

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 bioproceses 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.