Minireview

Using gas mixtures of CO, CO$_2$ and H$_2$ as microbial substrates: the do’s and don’ts of successful technology transfer from laboratory to production scale

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Summary

The reduction of CO$_2$ emissions is a global effort which is not only supported by the society and politicians but also by the industry. Chemical producers worldwide follow the strategic goal to reduce CO$_2$ emissions by replacing existing fossil-based production routes with sustainable alternatives. The smart use of CO and CO$_2$/H$_2$ mixtures even allows to produce important chemical building blocks consuming the said gases as substrates in carboxydotrophic fermentations with acetogenic bacteria. However, existing industrial infrastructure and market demands impose constraints on microbes, bioprocesses and products that require careful consideration to ensure technical and economic success. The mini review provides scientific and industrial facets finally to enable the successful implementation of gas fermentation technologies in the industrial scale.

Introduction

Since at least December 2015, when an overwhelming majority of nations worldwide agreed on signing the Paris Climate Agreement, scientific theories of climate change attributing the increasing man-made release of CO$_2$ a dominating role started to drive political and economic decision-making (Philip, 2018). Further supported by the inherent fear of diminishing fossil resources, oil-based chemical industries worldwide began to develop future scenarios for ensuring the current product portfolios, aiming for zero-CO$_2$ emission strategies. At best, future processes should not only prevent non-necessary CO$_2$ emissions, preferred production technologies should even incorporate CO$_2$ (Bengelsdorf and Dürr, 2017), thereby contributing to the climate goals and preventing costly payments for CO$_2$ certificates.

One strategy to prevent CO$_2$ emissions is the implementation of the so-called circular economy, that is the use of sugar contents in lignocellulosic feedstocks such as agri-residues, agri-processing by-products and energy crops for the microbial production of value-added products such as biofuels or fine chemicals (Liguori and Faraco, 2016). Expectations formulated in the US Energy Independence and Security Act (2007) specified 35 billion gallons of ethanol equivalents to be used in 2022 as a strategic goal. However, the technically and economically successful implementation of such processes has revealed to be very challenging, still requiring progress. Handling and use of the lignin fraction including energy management is one of the key challenges.

Biomass gasification either conventional (Griffin and Schultz, 2012) or via fast pyrolysis (Pfitzer et al., 2016; Arnold et al., 2017) is very well suited to use lignin-
containing sources such as wood or even municipal wastes for the production of CO and H2-containing gases, further called ‘syngas’ for simplification (LanzaTech, 2017). Such compositions vary between 30–60% CO, 25–30% H2, 0–5% CH4, 5–15% CO2, may contain other impurities such as H2S, NH3 and depend heavily on the source. A key transformation is the so-called water/gas-shift (WGS) reaction that converts CO and H2O into CO2 and H2 under high pressure and high temperatures (> 600°C) thus representing a source of H2 production. Besides for heat and power supply, syngas is applied in Fischer–Tropsch (FT) synthesis for the production of naphtha-like mixtures, diesel, methanol or even ethanol. However, such processes are not only energy-intensive (20–300 bar, 200–350°C), and they also require the removal of impurities like tars to protect the Rh catalysts. Furthermore, optimum FT conversions are only achieved if the preferred ratio H2 to CO of > 2 is installed (Abu-backar et al., 2011; Griffin and Schultz, 2012).

Interesting enough, nature provides a whole bunch of fermentative microbes that may grow on H2, CO and CO2 compositions via hydrogenesis, methanogenesis or acetogenesis (Latif et al., 2014; Diender et al., 2015). The latter are particularly promising for industrial application as they make use of the reductive acetyl-CoA pathway (Dürre, 2016). In a nutshell, CO and H2 serve as electron donors enabling the growth on CO2 and H2, or CO, or CO and H2 to produce mixtures of acetate, ethanol, 2,3 butanediol, etc. (Daniell et al., 2012). Accordingly, not only syngas, but also other off-gas compositions, for example from coke oven plants and steel industry, may provide valuable electron sources for the microbes. Thereby, the CO dehydrogenase (CODH) is the enabling enzyme and the biological equivalent to the harsh technical approach, however, working under moderate, anaerobic conditions. Furthermore, feasible H2/CO ratios are much more flexible than in Fischer–Tropsch (FT) processes (Munasinghe and Khanal, 2010), albeit cellular performance might suffer from gas impurities such as sulfur dioxide or hydrogen sulfide. Nevertheless, such processes offer the potential to be economically superior to conventional FT approaches (Griffin and Schultz, 2012). Nevertheless, some inherent drawbacks still hamper the success of aceticogenic production processes. Engineering challenges are mirrored by the poor water solubilities and low Henry constants of CO and H2 which are about 30 and 1.6 mg l−1 (of pure gases), and 27.1 and 1.6 mg bar−1 respectively.

Aside from the anaerobic, aceticogenic bacteria, the aerobic carboxydrotrophic bacteria are promising candidates for microbial production of value-added products from gases. These organisms are able to grow chemolithoautotrophically on CO or syngas by use of the reductive pentose phosphate pathway for anabolism and O2 as final electron acceptor in energy metabolism. Aerobic CO oxidation is more exotherm and allows higher ATP generation than anaerobic fermentation with CO, and therefore, the production of complex and more ATP-intensive products should be feasible. However, molecular toolboxes for carboxydrotrophic bacteria are missing, and thus, strain engineering is also still challenging.

This review not only provides biological and technical fundamentals for using aceticogenic or aerobic, carboxydrotrophic bacteria in gas fermentation, it also outlines the industrial point of view integrating such bioprocesses into both existing infrastructure and existing value-added chains (VACs). Thereof, conclusions will be drawn to make zero-CO2 initiatives an environmental and economic success.

The status quo in chemical industry

The production landscape in chemical industry is often organized in value-added chains (VACs), each leading from basic raw materials to molecules of interest via specific, often multifunctional intermediates. Currently, most VACs are based on fossil raw materials deploying homogeneous and heterogeneous chemical catalysis as major synthesis technologies. However, driven by competitiveness and the mindset of zero-CO2 emission processes, the use of alternative raw materials, such as renewable raw materials (RRM), is gaining importance.

Nevertheless, such processes necessarily need to offer competitive products, produced via efficient approaches delivering material with at least the same quality and performance as produced from fossil resources. Irrespective of the raw material used and the chemical or biochemical conversions applied, the following constraints need to be fulfilled for each industrial process:

(i) New processes to produce existing products (drop-in) need to show the potential to reach the efficiency and economic performance of mature traditional production routes;
(ii) Product and process specifications need to consider the variability in raw material and the consequences of biosynthesis;
(iii) Methods, techniques and equipment to economically handle large amounts of aqueous systems and purify molecules of interest in an aqueous environment are indispensable;
(iv) In the long run, and taking into account both, economic reality and depreciation of existing assets, current VACs might need to be altered to take full advantage of bio-based processes which might lead to new molecules.

On the other hand, gaseous substrates such as H2, CO and CO2 offer general benefits such as global
availability, large capacities, no interference with food or feed (based on gas generation from fossil resources or wastes), predictable pricing through well-known mechanisms, full metabolic usability, potentially less impurities derived from non-reactive carbon, good storability, reduced risk of infection, access from waste streams and enabler for circular economy considerations.

**Products of interest**

Driven by the inherent potential of gas fermentation, chemical industry should have a key interest to evaluate gaseous substrates for production purposes for intermediates as well as for performance molecules. Examples of the first are compounds such as 3-hydroxypropionic acid, succinic acid, itaconic acid, 1,4-butanediol, isobutene, 1-octanol, methyl methacrylate (MMA), butadiene, fatty acids, amino acids. Examples of the second are highly functionalized short- and medium-chain molecules, active pharma ingredients, vitamins, industrial enzymes, proteins, etc. It is particularly the first group that feels the strongest market pressure of success which is mirrored by the competition with traditional, fossil-based production technologies, the high cost pressure and strict specifications given by chemical VACs and applications. On the other side, physicochemical properties of the compounds are often well known which offers a broad range of feasible downstream processing (DSP) unit operations, including non-aqueous media in medium- to large-size production volumes.

To be successful, intermediates and performance molecules need to fulfill different criteria which mirror the individual production and market scenarios. Given the typically large market volumes of intermediates, also called commodities, of > 100,000 tons per year, maximum product concentrations (>100 g kg⁻¹), production rates (> 4 g kg⁻¹ h⁻¹), conversion and downstream processing yields, and highest product purity are crucial properties. On the other hand, high-process flexibility and product innovation levels are characteristic for performance molecules (see Fig. 1). As a consequence, gaseous substrates are particularly attractive for the production of commodities and are in the focus of current studies.

**Economic constraints of attractive gaseous substrates**

The economic success of a gas fermentation-based process will depend on several factors. First and foremost, the nature of the gaseous substrate is key. Gas mixtures of CO, CO₂ and H₂ are favourable due the inherent energy content of CO and H₂. Off-gases containing exclusively CO₂ (> 95%) are less attractive, however, available in large amounts from power plants or large-scale fermentations (e.g. bioethanol production). Unlike

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**Fig. 1.** Crucial properties of success are given for intermediates of VACs (indicated as small molecules) and performance molecules (indicated as industrial enzymes). The ranking from 0 to 10 represents a qualitative measure.

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syngas, CO₂-rich waste streams are inert and CO₂ activation requires reductive energy. Therefore, economic viability is crucially dependent on the availability of cheap and renewable electricity which has to be considered as the other feedstock besides CO₂.

Hydrogen is a versatile energy transport molecule that can be stored using existing technologies (compression, hydrogen—gas grids). Currently, hydrogen formation by the alkaline technology or by the more preferable Proton-Exchange-Membrane (PEM) technology is still not cost competitive to the alternative Steam-Methane-Reformation (SMR) process that uses natural gas (methane). The latter liberates CO₂, still, in some cases, the stoichiometry of the overall production process allows for a net fixation of CO₂. In these cases, SMR can serve as a bridging technology towards a fully renewable process that is based on the flexible PEM technology. PEM hydrolyzers are costly, but prices are expected to fall by more than 30% within the next years (Bertuccioli et al., 2014). Considering that electricity prices dominate hydrogen—feedstock costs, product formation from CO₂ will be most attractive during periods of surplus wind- and solar energy. Coupling of the energy providing industries with chemical industries (sector coupling) will be of increasing importance. However, existing regulatory frameworks have to be considered which might hamper cross-industry synergies.

Molecular oxygen is the other product of the water splitting reaction and will be available in high purities and in large amounts, thus credits from O₂ production can lower the hydrogen production costs. The often asynchronous availability of the three gases CO₂, H₂, and O₂ asks for smart gas storage systems, but compressing gases is an established, however, energy-demanding technique. Again, availability of cheap energy enters centre stage.

The common notion about CO₂ is a valueless waste stream instead of a valuable feedstock. The revenues from the European Emissions Trading System (ETS certificates, about 7 €/ton) currently underpin this notion. They are too low to have a positive impact on business models that aim for CO₂ upgrading (Pérez-Fortes et al., 2016). However, depending on its purity, CO₂ already has some markets mainly in food industries (beverages, green houses), thereby generating revenues with comparably little need of costly upfront investments (CAPEX, e.g., for gas stripping and compression technologies). CO₂ finds further applications as co-polymer in chemical synthesis, as shielding gas, extinguishing agent and as coolant. Existing CO₂ markets compete for the feedstock with the novel VACs to be established. However, CO₂ availability at point sources (steel mills, power stations, large-scale biological fermentations) outnumbers the current demand by orders of magnitude (Mikkelsen et al., 2010). Accordingly, low CO₂ feedstock prices will stay rather stable in the foreseeable future. For economic reasons, point sources of CO₂ should be located at sites where renewable energy is available in sufficient amounts, for example Iceland (geothermal energy), Morocco (solar) or Norway (wind). Liquidification of CO₂ is an established technology, and long-distance transport is economically viable for other gases (e.g. LNG, liquified natural gas). Unlike syngas, CO₂ is available from the atmosphere in almost unlimited quantities. Average annual anthropogenic CO₂ emissions are in the range of 35 - 40 Gt, and CO₂ emissions from fossil fuels and industry are expected to grow (Le Quéré et al., 2017). Consequently, direct air-capture of CO₂ in combination with gas fermentation at preferred sites becomes an interesting future option to enable economically viable production processes at currently unrecognized places. First pilot and commercial processes for direct air-capture have been implemented but lack economic competitiveness at present (Climeworks, Skytree). With regard to syngas, gas fermentation facilities will have to be located next to the syngas-emitting point source, and the first pilot- and demonstration-scale processes are implemented (Lanzatech, 2017).

The industrial wish list of microbial properties

Any microbial strain used in industrial applications needs to meet basic expectations. Properties such as high product tolerance, robustness with respect to harsh production conditions, genetic stability and high substrate uptake and conversion rates are highly appreciated aside from the expected high conversion yields. Regarding gas fermentations, the wish list can even be extended: the gaseous substrate might contain oxygen along with other trace toxins (e.g. sulfide) finally accumulating in the nutrient broth. Accordingly, oxygen tolerance is desirable, although somewhat contradicting with the native endowments of anaerobic acetogenic bacteria that are often applied. Similarly, microbes should tolerate elevated substrate levels (CO₂, H₂, CO), which are often mandatory in technical processes. Also, pH drops should be accepted because acid production typically coincides with the production of the product of choice.

Fermentative metabolism of CO, H₂ and CO₂ containing gases

Anaerobic acetogenic bacteria use the so-called Wood–Ljungdahl pathway for fixation of CO₂ or CO (Bengelsdorf et al., 2018). This pathway consists of two parts: the methyl and the carbonyl branch. In the former, a molecule of CO₂ is reduced to formate (in case of CO as a carbon source, this is first oxidized to CO₂) (Fig. 2).
Formate is coupled to the coenzyme tetrahydrofolate, thereby hydrolysing one ATP into ADP and inorganic phosphate. Then, the C1 unit is successively reduced to methyl-tetrahydrofolate, and the methyl group is transferred to an iron–sulfur–corrinoid protein. In the carbonyl branch, a molecule of CO2 is reduced to CO, using reduced ferredoxin, by the enzyme acetyl-CoA synthase/CO dehydrogenase, which then also combines the methyl group of the iron–sulfur–corrinoid protein, a CoA moiety and the carbonyl group into acetyl-CoA. This intermediate is further metabolized into acetate, yielding one ATP in the acetate kinase reaction. Thus, no substrate level-phosphorylated ATP is left for growth and biosynthetic reactions. Acetogens that produce ethanol in addition to acetate do so using an aldehyde: ferredoxin oxidoreductase, which converts acetate and reduced ferredoxin to acetaldehyde that, in a further enzymatic reaction, is reduced to ethanol. This way, the bacteria are still able to generate ATP from acetate formation. Reducing equivalents are usually produced by bifurcating hydrogenases that oxidize two H2 and reduce both, ferredoxin and NAD⁺.

Numerous acetogens are currently known (Table 1). However, only few serve as model organisms and industrial workhorses, that is Acetobacterium woodii, Clostridium ljungdahlii, Moorella thermoacetica and Clostridium autoethanogenum. Although the current scientific and industrial interest mostly focuses on the autotrophic features, it must be mentioned that all acetogens known are also able to use a number of heterotrophic substrates. A. woodii was isolated in 1977, when the very first described autotrophic acetogen, that is C. aceticum was still considered to be lost (Balch et al., 1977).

C. aceticum was only rediscovered in 1980 and later.
| Organism                        | Substrate               | Products/Methanol utilization (yes or no) | Optimal growth temperature [°C] | Optimal pH | Doubling time (autotrophic) [h] | Genome accession number | References                                      |
|--------------------------------|-------------------------|------------------------------------------|---------------------------------|------------|--------------------------------|-------------------------|------------------------------------------------|
| Acetobacterium ruminis DSM 5522| H₂ + CO₂, CO            | Acetate/no                               | 37–42                           | 6.8        | H₂ + CO₂: 2.1                  | FOJY0000000000          | (Greening and Leedle, 1989)                      |
| Acetobacterium baktii DSM 8239 | H₂ + CO₂                | Acetate/no                               | 37                              | 7.6–7.8    | H₂ + CO₂: 27                  | FUYNO0000000000         | (Sleat et al., 1985)                             |
| Acetobacterium carbinolicum DSM | H₂ + CO₂                | Acetate/yes                              | 20                              | 6.5        |                                |                         | (Kotsyurbenko et al., 1995)                      |
| Acetobacterium malicum DSM 4132| H₂ + CO₂                | Acetate/yes                              | 27                              | 7.0–7.2    |                                |                         | (Eichler and Schink, 1984; Schuppert and Schink, 1990) |
| Acetobacterium fi metarium DSM 8237| H₂ + CO₂, CO           | Acetate/no                               | 30                              | 7.5        |                                |                         | (Kotsyurbenko et al., 1995)                      |
| Acetobacterium tundrae DSM 9173| H₂ + CO₂                | Acetate/yes                              | 20                              | 7.0        |                                |                         | (Tanaka and Pfennig, 1988)                       |
| Acetobacterium woodii DSM 1030 | H₂ + CO₂                | Acetate/no                               | 20                              | 7.0        |                                |                         | (Kotsyurbenko et al., 1995)                      |
| Acetoclasticum arabaticum DSM 5501| H₂ + CO₂, CO            | Acetate/no                               | 38–40                           | 7.6–8.0    |                                | CP002105                | (Simankova et al., 2000)                         |
| Alkalibaculum bacchi DSM 6540  | H₂ + CO₂                | Acetate, butyrate/n.r.                   | 30–33                           | 7.8        |                                |                         | (Braun and Gottschalk, 1982; Poehlein et al., 2016) |
| Alkalibaculum bacchi DSM 22112| H₂ + CO₂                | Acetate, CO₂, ethanol/yes                | 37                              | 8.0–8.5    |                                |                         | (Zhilina and Zavarzin, 1990; Sikorski et al., 2010) |
| Butyribacterium methylotrophicum DSM 3468 | H₂ + CO₂, CO (after adaption) | Acetate, ethanol, butyrate, butanol/yes | 37–40                           | 7.5        | CO: 13.9                      | MIMZ0000000000         | (Zeikus et al., 1980; Lynd et al., 1982) Bengelsdorf et al., 2016b) |
| Clostridium acetircum DSM 1496 | H₂ + CO₂                | Acetate/yes                              | 30                              | 8.3        | H₂ + CO₂: 20-25               | CP009687-CP009688       | (Wieringa, 1936; Lux and Drake, 1984) Bengelsdorf et al., 2016b) |
| Clostridium autoethanogenum DSM 10061 | H₂ + CO₂                | 2,3-butanediol, acetate, ethanol/no     | 37                              | 5.8–6.0    | CO: 4                         | CP006763                | (Abrini et al., 1994; Köpke et al., 2011) Brown et al., 2014) |
| Organism                              | Substrate                  | Products/Methanol utilization (yes or no) | Optimal growth temperature [°C] | Optimal pH | Doubling time (autotrophic) [h] | Genome accession number | References |
|--------------------------------------|----------------------------|------------------------------------------|--------------------------------|------------|---------------------------------|-------------------------|------------|
| Clostridium carboxidivorans DSM 15243 | H₂ + CO₂, CO              | Acetate, ethanol, butyrate, butanol/no   | 38                             | 5.0–7.0    | CO: 6.3, H₂ + CO₂: 8.3          | CP011803-CP011804       | (Liou et al., 2005) |
| Clostridium coskattii ATCC PTA-10522 | H₂ + CO₂, CO              | Acetate, ethanol/no                      | 37                             | 5.8–6.5    |                                 | LROR00000000            | (Zahn and Saxena, 2012 Bengelsdorf et al., 2016a; Köpke et al., 2013 Riedel et al., 2015) |
| Clostridoides difficile DSM 27543    | H₂ + CO₂                  | Acetate/n.r.                             | 37①                           | 5.9①       |                                 | CP010905                | (Küsel et al., 2000; Liou et al., 2005; Gößner et al., 2008; Jeong et al., 2014) |
| Clostridium drakei DSM 12750         | H₂ + CO₂, CO              | Acetate, ethanol, butyrate/no            | 30–37                          | 5.4–7.5    | CO: 8.3, H₂ + CO₂: 5.0          | JIBU02000000            |           |
| Clostridium formicaceticum DSM 92    | CO                        | Acetate, formate/yes                     | 37                             | 8.1        | CO: 10                          | CP020559                | (Andreesen et al., 1970; Lux and Drake, 1992 Karl et al., 2017) |
| Clostridium ljungdahlii DSM 13528    | H₂ + CO₂, CO              | 2,3-butanediol, acetate, ethanol/no      | 37                             | 6.0        | CO: 3.8                         | CP001666                | (Schink, 1984; Schink, 1991; Uhlig et al., 2016) |
| Clostridium magnum DSM 2767          | H₂ + CO₂                  | Acetate/yes                              | 30–32                          | 7.2        |                                 | ATXD01000000            | (Mechichi et al., 1999) |
| Clostridium scatologenes DSM 757     | H₂ + CO₂, CO              | Acetate, ethanol, butyrate/no            | 37–40                          | 5.4–7.0    | CO: 11.1, H₂ + CO₂: 25.0        | CP009933                | (Liou et al., 2005; Zhu et al., 2015) |
| Desulfitomaculum thermobenzoicum subsp. thermosyntrophicum DSM 14055 | H₂ + CO₂                  | Acetate, formate/yes                     | 35                             | 7.2        |                                 | FNRK00000000            | (Mechichi et al., 1998) |
| Eubacterium aggregans DSM 12183      | H₂ + CO₂                  | Acetate, formate/yes                     | 39                             | 7.0–7.2    | CO: 7, H₂ + CO₂: 14             | CP019962                | (Plugge et al., 2002) |
| Fuchsiella alkaliacetigena DSM 24880 | H₂ + CO₂                  | Acetate/yes                              | 40                             | 8.8–9.3    |                                 |                        | (Zhilina et al., 2011) |
| Fuchsiella ferrireducens DSM 26031    | H₂ + CO₂                  | Acetate/yes                              | 30–37                          | 9.8        |                                 |                        | (Zhilina et al., 2015) |
| Holophaga foetida DSM 6591           | N.r.                      | N.r./no                                  | 28–32                          | 6.8–7.5    |                                 | AGSB02000000            | (Liesack et al., 1994 Anderson et al., 2012) |
| Marvinbryantia formatexigens DSM 14469 | H₂ + CO₂, formate          | Acetate/yes                              | 37①                           | 7.0①       |                                 | ACCL00000000            | (Wolin et al., 2003, 2008) |
| Oxobacter pfennigii DSM 3222         | H₂ + CO₂, CO              | Acetate, butyrate/no                     | 36–38                          | 7.3        | CO: 13.9                        | LKET01000000            | (Krumholz and Bryant, 1985; Bengelsdorf et al., 2015b) |
| Sporomusa acidovorans DSM 3132       | H₂ + CO₂                  | Acetate/yes                              | 35                             | 6.5–7.0    |                                 | LSSL00000000            | (Ollivier et al., 1985 Humphreys et al., 2017a) |
| Sporomusa aerivorans DSM 13326       | H₂ + CO₂                  | Acetate/yes                              | 30                             | 7.0        | H₂ + CO₂: 8.9                    | FWX10000000            | (Boega et al., 2003) |
| Sporomusa malonica DSM 5090          | H₂ + CO₂                  | Acetate/yes                              | 28–32                          | 7.3        |                                 |                        | (Dehning et al., 1989) |
| Organism                        | Substrate          | Products/Methanol utilization (yes or no) | Optimal growth temperature [°C] | Optimal pH | Doubling time (autotrophic) [h] | Genome accession number | References                                                                 |
|--------------------------------|--------------------|------------------------------------------|---------------------------------|----------|-------------------------------|------------------------|---------------------------------------------------------------------------|
| *Sporomusa ovata* DSM 2662     | H₂ + CO₂           | Acetate/yes                               | 34                              | 6.3      |                               | ASXP010000008           | (Moeller et al., 1984; Poehlein et al., 2013)                              |
| *Sporomusa paucivorans* DSM 3697| H₂ + CO₂           | Acetate/yes                               | 34                              | 6.7      | H₂ + CO₂: 10                  |                        | (Hermann et al., 1987)                                                   |
| *Sporomusa rhizae* DSM 16652   | H₂ + CO₂           | Acetate/yes                               | 35                              | 7.5      |                               |                        | (Goetner, 2006)                                                         |
| *Sporomusa silvacetica* DSM 10669| H₂ + CO₂           | Acetate/yes                               | 25—30                           | 5.5—7.7  |                               | LSLK00000000            | (Kuhner et al., 1997; Humphreys et al., 2017b)                           |
| *Sporomusa sphaeroides* DSM 2875| H₂ + CO₂           | Acetate/yes                               | 35—37                           | 6.5      |                               | LSLJ00000000            | (Moeller et al., 1984; Castilo et al., 2017)                              |
| *Sporomusa termitida* DSM 4440 | H₂ + CO₂, CO       | Acetate/yes                               | 30                              | 7.2      | H₂ + CO₂: 7.8                 |                        | (Breznak et al., 1988)                                                   |
| *Terrisporobacter glycolicus* RD-1 | H₂ + CO₂           | Acetate/yes                               | 37—40                           | 7.0—7.5  |                               | AUUB01000000            | (Kusel et al., 2001; Gerritsen et al., 2014)                               |
| *Terrisporobacter mayombei* DSM 6539| H₂ + CO₂           | Acetate/yes                               | 33                              | 7.3      | H₂ + CO₂: 5                   |                        | (Kane et al., 1991; Gerritsen et al., 2014)                               |
| *Treponema primitia* DSM 12427 | H₂ + CO₂           | Acetate/yes                               | 30                              | 7.2      | H₂ + CO₂: 29                  | CP001843               | (Grabar et al., 2004; Graber and Breznak, 2004; Rosenthal et al., 2011) |
| *Calderihabitans maritimus* DSM 26464| CO               | H₂ + CO₂ acetate/no                      | 65                              | 7.0—7.5  |                               | BDGJ00000000            | (Yoneda et al., 2013; Omae et al., 2017)                                  |
| *Carboxydotermus ferrarireducens* DSM 11255| H₂ + CO₂, CO     | N.r./no                                   | 65                              | 6.0—6.2  |                               | ATYG00000000            | (Slobodkin et al., 1997; Slobodkin, 2006)                                  |
| *Carboxydotermus* hydrogenotrans DSM 6008 | CO               | H₂ + CO₂/no                               | 70—72                           | 6.8—7.0  | CO: 2                         | CP00141                | (Svetlichny et al., 1991; Wu et al., 2005)                                 |
| *Carboxydotermus pertinax* DSM 23698| H₂ + CO₂           | H₂ + CO₂/no                               | 65                              | 6.0—6.5  | CO: 1.5                       | BDJK00000000            | (Yoneda et al., 2012; Fukuyama et al., 2017)                               |
| *Moorella glycerini* DSM 11254 | N.r.               | Acetate/n.r.                              | 58                              | 6.3—6.5  |                               | CELZ00000000            | (Slobodkin et al., 1997)                                                  |
| *Moorella maderi* DSM 14980    | H₂ + CO₂           | Acetate/yes                               | 65                              | 7.0      |                               | LTBC00000000            | (Balk et al., 2003; Castilo et al., 2016)                                  |
| *Moorella thermoacetica* DSM 2955| H₂ + CO₂, CO      | Acetate/yes                               | 55                              | 6.9      | CO: 9—16                      | CP012369               | (Fontaine et al., 1942; Kerby and Zeikus, 1983; Andresen et al., 1973; Daniel et al., 1990; Parekh and Cheryan, 1991; Goetner et al., 1999; Bengelsdorf et al., 2015a; Poehlein et al., 2015a) |
| *Moorella thermoaurotrophica* DSM 1974| H₂ + CO₂, CO      | Acetate/yes                               | 56—60                           | 5.7      | H₂ + CO₂: 8                   |                        | (Wiegel et al., 1981)                                                   |

n.d., not deposited; n.r., not reported.

a. No type strain.

b. Condition not described as optimal, but used in the reference.

c. No validly described species.

d. No growth on gas reported, but all Wood–Ljungdahl pathway genes found in the genome.

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completely sequenced (Braun et al., 1981; Poehlein et al., 2015a,b,c). A. woodii does not use CO as a substrate, but is very efficient in fermenting CO₂/H₂ gas mixtures. It is now considered as the model acetogen for sodium bioenergetics. Energy conservation is based on a Rnf complex that exports Na⁺ ions and a sodium-dependent ATPase that imports Na⁺ ions for ATP generation (Müller et al., 2001; Biegel and Müller, 2010; Hess et al., 2013). The organism is genetically accessible since more than 20 years (Strätz et al., 1994), the complete genome sequence is known (Poehlein et al., 2012). A. woodii can be grown in bioreactors with continuous gas supply, and recombinant strains for non-natural product formation (acetone) have been constructed and tested (Straub et al., 2014; Hoffmeister et al., 2016; Kantzow and Weuster-Botz, 2016).

Clostridium ljungdahlii has been isolated for its ability to grow on CO and CO-containing gas mixtures such as syngas (Tanner et al., 1993). CO₂/H₂ gas mixtures cannot be utilized as efficiently as by A. woodii. C. ljungdahlii also relies on a Rnf complex for generation of an ion gradient, but in this case, it is proton-dependent (Tremblay et al., 2012) and thus coupled to a H⁺-dependent ATPase. The organism is also genetically well accessible and completely sequenced. Recombinant strain construction (butanol) has been achieved as well (Köpke et al., 2010). C. ljungdahlii is meanwhile considered to be a model acetogen for proton bioenergetics and CO utilization.

A very close relative, C. autoethanogenum, was described only a few months after C. ljungdahlii (Abrini et al., 1994). Phylogenetically, it contains an identical 16S rRNA gene as C. ljungdahlii and both show a very high genome sequence similarity, however, no identity (> 98%; Humphreys et al., 2015). Its characteristics are very comparable to C. ljungdahlii, with only few differences (Humphreys et al., 2015). C. autoethanogenum was meanwhile developed into the industrial acetogen workhorse, being employed by the leading company in this field, LanzaTech, Inc (Skokie, IL, USA).

Finally, M. thermoacetica is a thermophilic acetogen, which was used as model organism for elucidation of the Wood–Ljungdahl pathway. Ironically, this was all performed with sugar-grown cultures, as CO-dependent growth was only found much later (Daniel et al., 1990). Besides being the best characterized acetogenic thermophile, M. thermoacetica also uses a different bioenergetic system. The organism possesses an Ech complex (no Rnf) and, in addition, also cytochromes and menaquinone (Gottwald et al., 1975; Das and Ljungdahl, 2003; Pierce et al., 2008; Schuchmann and Müller, 2014). Thus, there are two potential possibilities to generate a proton gradient across the membrane.

### Aerobic metabolism of CO, H₂ and CO₂ containing gases

Whereas anaerobic fermentation of CO, H₂ and CO₂-containing gases with acetogenic bacteria is well known and already employed for industrial ethanol production, the aerobic utilization of such gases for biotechnological purposes is still in its infancy and has not been exploited so far. This certainly is due to the fact that in spite of numerous organisms able to grow chemolithoautotrophically on H₂, CO₂ and CO mixtures (King, 2003; King and Weber, 2007), only few of these so-called carboxydotrophic bacteria have been characterized in detail, molecular toolboxes for these organisms have not been developed, H₂ and CO show very low solubility in aqueous solutions, CO is highly toxic, and the handling of H₂ and CO in the presence of O₂ requires extensive precautions. However, aerobic CO oxidation is energetically more favourable than anaerobic oxidation and subsequent acetogenesis (reactions 1 and 2, respectively; Diender et al., 2015) and, thus, aerobic oxidation of CO should allow the production of more costly (i.e. ATP-intensive) products than CO oxidation via anaerobic acetogenesis.

**Reaction I** : 2 CO + O₂ → 2 CO₂ \((\Delta G^0 = -514 \text{kJ})\)

**Reaction II** : 4 CO + 2H₂O → CH₃COO⁻ + H⁺ + 2 CO₂ \((\Delta G^0 = -174 \text{kJ})\)

Accordingly, bioreactors for aerobic, carboxydotrophic cultivation need to be equipped with sufficient cooling capacities to buffer the heat release caused by necessarily tightly controlled H₂ oxidation \((\Delta H^0 = 286 \text{kJ mole}^{-1})\) which somewhat resembles the cooling demands of comparable sugar-based, aerobic scenarios.

Species of Oligotropha, Bradyrhizobium, Mesorhizobium, Hydrogenophaga, Burkholderia and also some species of Mycobacterium, Pseudomonas, Alcaligenes and Acinetobacter have been reported to grow aerobically on CO and CO- and H₂-containing gases as sole carbon and energy sources (reviewed in Meyer and Schlegel, 1983; King, 2003; King and Weber, 2007; Weber and King, 2012). Especially, carboxydotrophic bacteria such as Oligotropha carboxidovorans and Hydrogenophaga pseudoflava which possess a highly CO-tolerant respiratory chain and show high growth rates under autotrophic conditions are promising candidates for future biotechnological application in aerobic gas fermentations (Zavarzin and Nozhevnikova, 1977; Cypionka et al., 1980; Cypionka and Meyer, 1982). Oligotropha carboxidovorans probably is the best studied carboxydotrophic bacterium. It possesses a CO-insensitive
aerobic electron transport chain, an O2-tolerant molybdenum and copper-containing CO dehydrogenase for oxidation of CO2 and the Calvin–Benson–Bassham cycle for fixation of CO2 during autotrophic growth on CO and CO2 (Meyer and Schlegel, 1978, 1983). O. carboxidovorans is also able to grow organoheterotrophically with organic acids (Meyer and Schlegel, 1983), its genome consists of one chromosome and two megaplasmids, one of which (pHCG3) harbours the (substrate-inducible) genes required for H2 and CO oxidation and for CO2 fixation (Fuhrmann et al., 2003; Paul et al., 2008; Volland et al., 2011). However, genetic tools are so far not available for this organism.

Very recently, Heinrich et al. (2017) reported on aerobic utilization of syngas by recombinant strains of Ralstonia eutropha H16 (currently designated as Cupriavidus necator H16). The wild type of this species is an aerobic and chemolithoautotrophic ‘Knallgas’ bacterium able to efficiently use H2 and CO2 as sole carbon and energy sources and possessing a hydrogenase and an electron chain which are relatively insensitive towards CO (Cyponka and Meyer, 1982; Friedrich and Schwartz, 1993; Bürstel et al., 2016). C. necator H16 was genetically engineered to express the genes encoding the O. carboxidovorans CO dehydrogenase as well as the genes encoding proteins for maturation of this enzyme. In the presence of H2, CO2 and CO (plus small amounts of a heterotrophic substrate in the preculture), the resulting strain was able to (slowly) oxidize and use CO as a carbon source, grew slightly faster and produced significantly more poly-D-3-hydroxybutyrate (PHB) than the parental strain carrying the empty plasmid on H2 and CO2 (Heinrich et al., 2017). Since R. eutropha has been shown to produce several recombinant higher-value products aside from PHB (reviewed in Dürre and Eikmanns, 2015), the metabolic engineering strategy applied seems very promising for further biotechnological exploitation.

The development of genetic tools for aerobic, carboxydotrophic bacteria is still in the beginning. However, genome analysis of promising representatives, establishment of sophisticated genetic engineering tools such as efficient transformation, differential expression of homologous and heterologous genes, markerless deletions and/or allelic exchanges should allow the construction of one or more carboxydotrophic (platform or model) organism/s for future biotechnological applications, that is for aerobic utilization of syngas for the production of value-added products.

**Bioprocess developments**

Autotrophic growth of acetogens is extremely energy limited and gas–liquid mass transport limitations restrict biocatalytic activities due to the low solubilities of the gaseous substrates H2 and CO in water at ambient pressure. H2 is 65 mol% and CO is 75 mol% less soluble in water compared to O2 (1 bar, 20 °C). Compared to typical aerobic heterotrophic bioprocesses, planktonic cell concentrations are reduced by a factor of 10 and more, resulting in low volumetric productivities of gas fermentations.

Gas–liquid mass transfer can be improved by increasing the volumetric power input into bioreactors with dispersed gas phase (e.g. increasing the stirrer speed in stirred-tank bioreactors) and/or increasing the partial pressures of H2 and CO in the gas phase. Increasing the power input per unit volume will cause severe scale-up challenges and will be economically demanding if low value adding products are produced from syngas like C2-C4 alcohols or acids. Increasing the partial pressures of H2 and CO will thus be the method of choice, for example by applying bioreactors with liquid heights h of 20–30 m to build up a high hydrostatic pressure at the bottom of the reactor where the syngas is dispersed. Amongst others, these are the reasons why bubble-column or gas-lift reactors are chosen for syngas fermentations with planktonic cells on an industrial scale. It must be pointed out that increased partial pressures of the gaseous substrates are favourable from a thermodynamic point of view as well.

The power input of bubble-column reactors on an industrial scale is caused by the isothermal expansion of the gas phase dispersed at the bottom of the reactor ($P_{Exp}$)

$$P_{Exp} = \frac{V_{gas} \cdot \rho_{gas}}{M_{gas}} \cdot \frac{RT}{\rho_U} \cdot \ln \left( \frac{1 + \rho_L \cdot g \cdot h}{\rho_U} \right), \quad (1)$$

with $V_{gas}$ as the inlet gas flow rate, $\rho_{gas}$ as the density of the gas, $M_{gas}$ as the molecular weight of the gas, $\rho_U$ as the pressure above the liquid surface (head-space pressure), $\rho_L$ as the density of the liquid phase, $g$ as the acceleration of gravity and $h$ as the liquid height above the gas sparger in the reactor. The power input $P_{Exp}$ increases with raising gas inlet flow rate and liquid height $h$. Due to the elevated partial pressures of the gases in the inlet at high liquid heights, increasing the liquid height $h$ results in elevated partial pressures at the bottom of the bubble-column reactor and increased power input both serving for improved gas–liquid mass transfer. As a consequence, solely acetogenic producer strains can be applied for syngas fermentation which are not inhibited by increased H2- or CO-partial pressures of up to a few bar. Unfortunately, H2- or CO-inhibition kinetics of acetogens are not very well studied so far (e.g. Vega et al., 1988; Chang et al., 1998; Skidmore et al., 2013; Mohammadi et al., 2014).
The partial pressures in the gas bubbles rising in a bubble column vary considerably as function of the height \( h \) in the column due to the consumption and production of gases by acetogens in the liquid phase and due to the decline of the total pressure. As a consequence, axial gradients are inevitable with respect to the partial pressures of the gas phase as well as the concentrations of biomass, products and pH in the liquid phase along the height of the bubble-column reactor. Whereas the multiphase transport processes can be described by well-known modelling approaches (e.g. plug flow of both phases with axial dispersion and gas–liquid mass transport in bubble columns), the gas consumption and product formation kinetics of acetogens as function of substrate (\( \text{H}_2, \text{CO} \)) and product concentrations (acetate, ethanol, etc.) as well as pH are not very well known. An approach to overcome this lack of kinetic information is making use of a genome-scale metabolic reconstruction of acetogens combined with uptake kinetics for \( \text{H}_2 \) and \( \text{CO} \) (e.g. Chen et al., 2015).

Further studies on the kinetics of acetogens in fully controlled and well-mixed laboratory-scale stirred-tank bioreactors are inevitable to provide the kinetic data which are needed for the modelling of syngas fermentation in bubble columns on an industrial scale. Batch syngas fermentation processes in stirred-tank bioreactors will provide basic performance data (e.g. Demler and Weuster-Botz, 2011, Groher and Weuster-Botz, 2016a,b; Kantzow and Weuster-Botz, 2016; Mayer and Weuster-Botz, 2017) but continuous syngas fermentations should be preferred due to the possibility to perform steady-state studies (e.g. Mohammadi et al., 2012). Low growth rates of acetogens especially if extreme reaction conditions are to be studied may be an obstacle (e.g. pH, T, inhibiting gas concentrations). The application of submerged microfiltration membranes in a continuously operated stirred-tank bioreactor enables the study of syngas fermentations with (total) cell retention (Kantzow et al., 2015). A cascade of stirred-tank bioreactors is another option with the first reactor operated at optimum autotrophic growth conditions for acetogens and the second reactor at extreme reaction conditions, for example for studying the reconsumption of acids and the production of alcohols at low pH without growth (Richter et al., 2013; Martin et al., 2015).

One of the first commercial plants for the conversion of CO-rich industrial off-gases of a steel mill is presently under construction at the ArcelorMittal steel mill in Ghent (Belgium) making use of continuously operated bubble-column/gas-lift loop reactors. The syngas fermentation process developed by LanzaTech uses Clostridium autoethanogenum for the production of ethanol with an estimated volumetric productivity of around 10 g l\(^{-1}\) h\(^{-1}\). The syngas fermentation plant in Ghent is designed for an annual production capacity of 60,000 m\(^3\) ethanol converting 50,000 Nm\(^3\) h\(^{-1}\) steel mill off-gases (Molitor et al., 2016) and is due to be operational in 2019. CO utilization of around 70–75% was shown at the Shougang (China) demonstration facility of LanzaTech (Heijstra et al., 2017). Current approaches even strive for almost complete CO utilization.

A promising alternative to bubble columns or gas-lift reactors may be trickle-bed biofilm reactors. A biofilm is formed on the (inner) surfaces of carrier materials forming a fixed-bed in a cylindrical reactor. The liquid phase is distributed above the fixed-bed and trickles down forming a thin liquid film on the surface of the carriers. In contrast to bubble-column reactors, the gas phase is not dispersed in the liquid phase but forms the continuous phase in the trickle-bed reactor. The pressure drop of the gas phase is thus negligible, and the power input of trickle-bed reactors is solely caused by pumping the liquid phase to the top of the fixed-bed of the trickle-bed reactor. Despite the low power input, the gas–liquid and gas-biofilm mass transfer becomes high at low liquid film thickness, high flow rates of the trickling liquid and low biofilm thickness. Due to the low operating costs at high mass transfer rates, trickle-bed biofilm reactors and a special design of thereof, so-called horizontal rotating packed-bed biofilm reactors, are extensively used in waste water treatment plants.

Very few studies have been reported so far on the application of trickle-bed reactors for syngas fermentation on a laboratory-scale (e.g. Bredwell et al., 1999; Yasin et al., 2015; Devarapalli et al., 2016; Schulte et al., 2016; Shen et al., 2017). High CO conversion rates of up to 91% were observed with Clostridium ragsdalei in a trickle-bed reactor with non-porous glass beads of 6 mm as carriers (Devarapalli et al., 2016). \( \text{H}_2 \) utilization of more than 80% was measured in a laboratory-scale horizontal rotating packed-bed biofilm reactor with Clostridium carboxidivorans P7 with non-porous HDPE carriers with a specific surface area of 500 m\(^2\) m\(^{-3}\). Compared to the syngas fermentation with a continuous operated stirred-tank bioreactor at the same operation conditions, the volumetric ethanol productivity was 3.3 times higher (Shen et al., 2017). Biofilm formation seems to be not an issue with acetogens although systematic studies are missing so far.

Axial gradients of pH and product concentrations are inevitable in the trickling liquid phase along the height of a trickle-bed reactor on an industrial scale (10 - 15 m). Recycling of the liquid phase may be a solution to reduce gradients but will increase the volumetric power input. Compared to bubble-column or gas-lift reactors, the liquid phase and biomass volume (‘working volume’) are very much reduced in trickle-bed reactors to about 20% of the total volume compared to about 80% in
bubble-column reactors. The reduced working volume of trickle-bed biofilm reactors will be balanced by improved mass transfer which is shown by higher volumetric productivities. However, industrial application of trickle-bed biofilm reactors for syngas fermentation will only be possible if a stable biofilm can be established for long-term operation, especially if recombinant acetogens will be applied for the (improved) production of natural or non-natural products. Unfortunately, no studies on control, stability and long-term operation of biofilms with (recombinant) acetogens have been published so far.

**Downstream processing**

‘Molecules don’t jump out of the broth’. The old wisdom is still valid, for commodities as well as for performance molecules. Given that 30–50% of manufacturing costs of commodities are typically assigned to downstream processing (DSP), processes may gain or lose their economic viability in the downstream section. In general, DSP aims at providing products that meet given purity specifications. However, specification sheets of VAC intermediates and other commoditized molecules are mainly derived from fossil-based production. Accordingly, listed impurities mirror the needs when dealing with fossil raw materials and do not cover impurity patterns of bio-processes. A basic change of mindset is necessary, because fossil-based impurities enter processes as co-substrates while bioprocesses typically produce non-wanted impurities via metabolic reactions. In consequence, specifications need to be revisited to fulfill their purpose of guaranteeing the performance of the respective product within the VAC and for the application. By-products of fermentation need to be integrated in ‘conventional’ specification lists checking whether or not their occurrence hampers the efficiency of subsequent processing steps or even the functionality of the final product. For instance, low impurity concentrations may cause non-wanted caking in DSP which in turn may necessitate repeated strain engineering to prevent by-product formation. The example outlines the crucial importance of mass balancing in DSP to track impurities. Furthermore, it shows that total bioprocess development is a workflow of different activities with distinct interfaces and feedback loops that needs to be cycled several times to succeed (Fig. 3).

**Intrinsic benefits of gas fermentation evaluated by conceptual design**

As outlined above, the minimization of manufacturing costs is key for the production of VAC intermediates and commodities. Accordingly, criteria shown in Figure 1 need to be optimized. Some helpful evaluation may even be performed during early-stage conceptual design using rather simple but characteristic assumptions. Considering a common sugar-based production capacity of 100 kilo-tons per annum (kta), average space–time yield (STY) of 4 g l⁻¹ h⁻¹, product titre of 100 g l⁻¹ (achieved after 40 h batch-time including turnaround), total product recovery of 85% and operation time of 8000 h a⁻¹, the following conclusion can be drawn: 200 batch cycles per annum will be necessary, cycling about 5882 m³ batch⁻¹. Consequently, 12 bioreactors, each ≥ 500 m³ working volume will be needed. However, gas fermentations offer the advantage to run in continuous mode which will extend ‘batch-times’ to 100 h. As a result, only 6 bioreactors ≥ 500 m³ working volume will be needed which illustrates the economic potential to save CAPEX (capital expenditure) and OPEX (operational expenditure) with gas fermentations. To improve the latter, gas fermentations typically consider operational pressures up to 4 bar for ensuring sufficient mass transfer and reasonable specific power input <0.3 kW m⁻³ in combination with loops to consume the gaseous substrates as good as possible.

**Size matters – large-scale production of intermediates**

Based on decades of experience within chemical process engineering, routines and short cut methods exist
to predict large-scale capital and operational expenditures. Such algorithms typically predict specific CAPEX decrease with increasing capacity because costs of apparatuses non-linearly increase with size. Beyond maximum apparatus size, number-up of equal devices is applied which keeps specific CAPEX constant. Such limits strongly depend on the devices used. For instance, solid-/liquid separation equipment such as centrifuges reaches the limit of scale-up much earlier than liquid processing equipment such as distillation columns.

The principle also holds true for different types of reactors. Continuous chemical reactor systems usually reach their maximum economy of scale at significantly higher capacities than batch-type bioreactors. Of course, this depends on process-specific performance indicators, that is space–time yield and catalyst-specific productivity. Accordingly, a fossil-based process to produce a commodity such as acrylic acid might reach its maximum economy of scale at capacities > 200 kt a⁻¹. In comparison, the fermentative production analogue of the respective precursor will get at the said limit already < 100 kt a⁻¹. In other words, bioprocesses accomplish economy of scale with less product than chemical counterparts.

The relation between CAPEX of a specific intermediate and the capacity of a respective bio-based process and production plant significantly depends on the ‘nature’ of the product and in consequence the efforts to be taken to deplete impurities. The lowest cost might be achieved with a low-boiling product that can be purified by distillation of the aqueous reaction mixture, for example ethanol. Also, gaseous products are advantageous in terms of DSP in case those could be purified via fractional condensation or pressure swing adsorption. Products which need to be handled as crystals demand higher specific CAPEX since solid-liquid separation and solids processing apparatus come at higher specific cost.

The perspective
As already outlined, gas fermentations using mixtures of CO, CO₂ and H₂ possess the inherent potential to substitute fossil-derived components by bio-based intermediates thereby continuing with already established VACs. CAPEX and OPEX scenarios look promising for production capacities > 100 kt a⁻¹ provided that product specifications are met. The successful solution of this challenge asks for improvements in strain and process engineering. Intrinsic problems of mass transfer and bioreactor design need to be solved which demands for the application of novel knowledge-based scale-up approach as already outlined in Takors (2012) and Delvigne et al. (2017). Besides, the economic access to electron-donating substrates (CO, H₂) is a prerequisite of every industrial process. Individual solutions may be found in highly networked composite chemical sites or via integration in future international energy grids.

CO₂, CO and H₂ were in the focus of this review, but alternatives such as CH₄ may be promising as well. The latter offers access to ATP-demanding product biosynthesis under aerobic conditions, thereby bypassing the intrinsic ATP limitations of anaerobic CO₂, CO and H₂ metabolism. However, aerobic carboxydrotroph strains may be another alternative requiring intensified research and sensitive large-scale engineering in compliance with ATEX regulations (ATmosphères EXPlosibles).

Currently, companies such as LanzaTech are succeeding to implement industrial scale gas fermentation for the production of native products such as ethanol or 2,3-butanediol. However, the turnaround from fossil raw materials to zero-CO₂ emission requires for much more VAC intermediates, as outlined above. The time is right to start research.

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Conflict of interest
None declared.

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