

Review Article

Single carbon metabolism – A new paradigm for microbial bioprocesses?

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ARTICLE INFO

Keywords:

Sustainability
Bioeconomy
Carbon dioxide
Methanol
Formate
Methane

ABSTRACT

Increasing atmospheric carbon dioxide levels, a reduction of arable land area and the dependence of first and second generation biotechnology feedstocks on agricultural products, call for alternative, sustainable feedstock sources for industrial applications. The direct use of CO₂ or conversion of CO₂ into other single carbon (C1) sources have great potential as they might help to reduce carbon emissions and do not compete with agricultural land use. Here we discuss the microbial use of C1 carbon sources, their potential applications in biotechnology, and challenges towards sustainable C1-based industrial biotechnology processes. We focus on methanol, formic acid, methane, syngas, and CO₂ as feedstocks for bioprocesses, their assimilation pathways, current and emerging applications, and limitations of their application. This mini-review is intended as a first introduction for researchers who are new to the field of C1 biotechnology.

1. Introduction

Rising atmospheric carbon dioxide (CO₂) levels are the main reason for the ongoing global climate crisis. In the year 2023 the record levels of more than 420 ppm of CO₂ were reached and the trend is still increasing [1] and global temperatures already increased by 1.1 °C (Fanning and Hickel, 2023; Morice et al., 2021). There is an urgent need for a reduction of greenhouse gas emissions and there are multiple agreements, most prominently the Paris Agreement, aiming to limit global warming to 2 °C compared to pre industrial levels [2]. A key point to fulfill these goals is to reduce or even stop the use of fossil fuels. Biotechnology could serve as a technology to help reduce the carbon footprint [3]. Amino acids, citric acid, and bio-ethanol production are examples where biotechnological processes are performed in large scales. Often these processes are based on substrates like sugars which are in direct competition with human consumption [4,5]. By using lignocellulosic biomass this competition can be reduced but complex pre-treatment steps of the biomass are necessary [6]. A possible future lies in single carbon (C1) feedstocks, which are either abundant in the air as CO₂ or can be produced from it, and so helping to reduce carbon emissions. This review gives an overview of main sources, microbial assimilation pathways and biotechnological use of selected C1 carbon sources, namely methanol, formic acid, CO₂, methane, and syngas. Single carbon molecules are interconvertible by oxidation and

reduction, respectively. Microbes make use of this to convert C1 substrates to two main precursors, formaldehyde and formate, which are the entry points into assimilation for most of the natural assimilation pathways (Fig. 1).

2. Methanol

Methanol is the simplest aliphatic alcohol. It is liquid at room temperature and can be used as starting material in chemical industry or fuel. A major drawback is the toxicity against humans and its flammability. Methanol was earlier produced as a by-product of the Fischer-Tropsch synthesis (FTS) using high temperatures and pressures using Cu–ZnO–Al₂O₃ or Cr₂O₃ based catalysts. Syngas used for FTS is often produced by steam reforming of natural gas or partial oxidation of residual oil [7,8]. Besides this classical production methods using fossil fuels where between 0.5 and 1.4 t CO₂eq/t MeOH are emitted depending on the starting material, there is a lot of research ongoing in the production of methanol using CO₂ as direct starting material [9]. Green methanol can be produced by using captured CO₂ from industries with high emissions and combining it with hydrogen produced by water electrolysis using green electricity [10]. Such a process has similar electrical but much lower thermal energy demands and a 3 times reduced carbon footprint [11]. Green methanol can as well be produced by direct electrochemical CO₂ reduction. This technology is still in

Peer review under responsibility of KeAi Communications Co., Ltd.

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E-mail address: oezge.ata@boku.ac.at (Ö. Ata).<https://doi.org/10.1016/j.synbio.2024.03.003>

Received 27 December 2023; Received in revised form 25 February 2024; Accepted 6 March 2024

Available online 11 March 2024

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development, especially faradaic efficiencies and current densities have to be improved [12,13].

Methanol can be used by methylotrophic bacteria like *Bacillus methanolicus* or *Methylobacterium extorquens* or yeasts like *Komagataella phaffii* (formerly *Pichia pastoris*) or *Ogataea polymorpha* (formerly *Hansenula polymorpha*). In bacteria methanol is mainly assimilated via either the serine cycle [14] or the ribulose monophosphate (RuMP) cycle [15], while yeasts employ the xylulose monophosphate (XuMP) cycle [16]. The first step of methanol utilization is its oxidation to formaldehyde which then enters one of the assimilation pathways mentioned above, either by a methanol dehydrogenase or by an alcohol oxidase. The oxidase reaction is thermodynamically favored (high negative delta Gibbs free energy: $\Delta G = -100$ kJ/mol) while dehydrogenases are energetically favorable as they harvest the energy of the oxidation reaction in the form of reduction equivalents (NAD^+ to NADH , or PQQ to PQQH_2). In short, dehydrogenase reactions have a less negative delta Gibbs free energy (PQQ dependent enzymes have a standard $\Delta G = -35$ kJ/mol, NAD^+ dependent enzymes have a standard $\Delta G = +30$ kJ/mol at ambient conditions) [17]. However, the disadvantage of the oxidase reaction is that the reduction equivalents (electrons) of the methanol oxidation are lost for the metabolism as they are fully transferred to oxygen, releasing the energy as metabolic heat instead of providing reduction equivalents to the cell.

Methylotrophic microorganisms have gained industrial interest in the 1960s first as a potential source of microbial protein for human nutrition and animal feed [18]. At that time cheap substrate sources originating from mineral oil and gas were envisioned. With the oil crisis in the mid 1970s these projects came to a halt. Research continued in different directions. Yeasts like *Komagataella phaffii* [19,20] and *Ogataea polymorpha* [21] were developed into production hosts for recombinant proteins when it was found that their alcohol oxidase genes were controlled by very strong and highly regulated promoters [22,23]. The success of these applications initiated further research on the fundamentals of methanol metabolism in these yeasts, leading to novel concepts of metabolic engineering to enhance productivity and carbon yield, or even expand the substrate spectrum [24–26]. Bacterial hosts [27], especially *M. extorquens*, were also used for the production of amino acids, dicarboxylic acids, mevalonates and their derivatives either by native or engineered strains [28]. Most methylotrophic

bacteria are however not well established as biotechnological production platforms, so that there may be a trade-off between methylotrophy and the productivity of a non-native product where native methanol consumers were engineered to overproduce chemicals [29]. To overcome the limitations related with low production yields, synthetic methylotrophy has been used as an approach in different host organisms which are widely used in biotechnology, such as *Escherichia coli* [26, 29–33], *Saccharomyces cerevisiae* [34–37] and *Corynebacterium glutamicum* [37–39]. So far, however, this has also not led to superior yields, probably limited by the fact that the engineered methanol utilization pathways are not fully balanced in their heterologous hosts. As an alternative approach, synthetic pathways also bear a huge potential with higher energetic efficiencies and production yields. This aspect is discussed in the last chapter (Synthetic C1 assimilation pathways).

3. Formic acid/formate

Formate has a low degree of reduction so that it is much less energy dense than methanol. Nevertheless, it can serve as a carbon and energy source for microorganisms, namely for different groups of prokaryotes.

Formate can enter metabolism via tetrahydrofolate (THF). Essentially two formate assimilation routes have been described, the serine cycle where a methylene group from methylene-THF is transferred to glycine, forming serine. With a co-assimilation of CO_2 towards oxaloacetate, glycine is finally recycled and one acetyl-CoA is released per cycle, assimilating one formate and one CO_2 . Alternatively, glycine can be synthesized *de novo* from methylene-THF and CO_2 via the reverse glycine cleavage system. With a second methylene-THF serine is formed, and then deaminated and reduced, leading to pyruvate which serves as a precursor for metabolites and biomass growth. Overall, one pyruvate is synthesized by the assimilation of two formate and one CO_2 . Formate utilization via the serine cycle is performed e.g. by *M. extorquens* [40] and other proteobacteria [41] while the reverse glycine cleavage system has been observed in nature only rarely, e.g. in *Desulfovibrio desulfuricans* [42] or *Clostridium drakei* [43] and recently also in the methylotrophic yeast *K. phaffii* [24,44]. Formatotrophic bacteria use formate not only as a carbon source, but gain their energy from oxidation of formate to CO_2 by NAD^+ dependent formate dehydrogenases, providing NADH as an electron donor for metabolic reactions and for the respiratory chain to

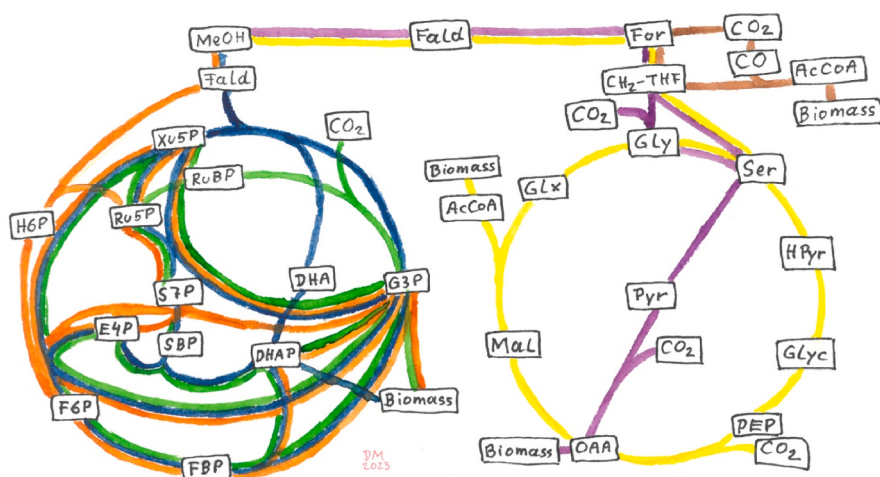


Fig. 1. Overview of the major native single carbon assimilation pathways. Methanol assimilation via formaldehyde is achieved by the RuMP cycle (orange) or the XuMP cycle (blue). These cycles bear high similarity to the CBB cycle for CO_2 assimilation (green). Formaldehyde can be further oxidized to formate and assimilated via the serine cycle (yellow) or the reductive glycine pathway (purple). The latter is not cyclic but a linear pathway, same as the Wood Ljungdahl pathway (brown). Abbreviations: AcCoA – acetyl coenzyme A, CH_2 -THF – methylene tetrahydrofolate, DHA – dihydroxyacetone, DHAP – dihydroxyacetone phosphate, E4P – erythrose 4-phosphate, Fald – formaldehyde, FBP – fructose biphosphate, For – formate, G3P – glyceraldehyde 3-phosphate, Gly – glycine, Glyc – glycerate, Glx – glyoxylate, H6P – hexulose 6-phosphate, Hpyr – hydroxypyruvate, Mal – malate, MeOH – methanol, OAA – oxaloacetate, PEP – phosphoenolpyruvate, Pyr – pyruvate, Ru5P – ribulose 5-phosphate, RuBP – ribulose biphosphate, S7P – sedoheptulose 7-phosphate, SBP – sedoheptulose biphosphate, Ser – serine, Xu5P – xylulose 5-phosphate.

produce ATP. Formate dehydrogenases are widely common in many organisms to detoxify formate, but also to serve for energy production in methylotrophic yeasts.

Formate is an interesting substrate for biotechnology: it is liquid and can be easily transported and stored at ambient temperatures and pressure, it is water miscible so that it can be easily mixed into large scale bioreactors at any desired concentration and rates. Not least, formate can be produced directly from CO₂ via electrochemistry, so that it may be regarded as one of the biotechnology substrates of the future. Electrochemical reduction of CO₂ to formate is technically quite advanced; however, scaling-up of the reduction process is still a challenge. Although there are upscaling attempts that reached within a range of 1–146 kg CO₂/day fixation and 12–110 kg formic acid/day, none of them are commercial yet [45,46]. Another limitation so far is in the choice of chassis organisms, as the native formatotrophs are rather exotic prokaryotes which are not used in biotechnology to date. Therefore, researchers have engineered formatotrophy into well established microorganisms like *E. coli*, *Pseudomonas putida* and yeast. By the integration of the reductive glycine pathway formatotrophic growth in *E. coli* was achieved reaching doubling times of 6 h [47]. Improved versions of formatotrophic *E. coli* strains were used for production of lactic acid but still reaching only low yields [48]. Also in *P. putida* which shows high tolerance against formate, formate assimilation via the reductive glycine pathway was demonstrated [49,50]. Lately a fully functional reductive glycine pathway was demonstrated in engineered *S. cerevisiae* [51].

4. Carbon dioxide

Atmospheric CO₂ levels are rising due to anthropogenic emissions. Re-use of CO₂ and its recycling into production processes are a top priority for an industrial transition. This means essentially to reduce the carbon and close carbon-carbon bonds. Microbial activity may offer various solutions to this end. While a huge surplus of CO₂ is freely available it is highly diluted in the atmosphere. To achieve higher concentrations CO₂ capture directly at industrial emitters may be the better choice.

Seven natural autotrophic carbon-fixation pathways have been identified to date. These pathways encompass the Calvin-Benson-Bassham (CBB) cycle [52], the reductive tricarboxylic acid cycle (rTCA) [53], the oxygen-sensitive Wood-Ljungdahl (WL) pathway [54], the 3-hydroxypropionate (3-HP) cycle [55], the hydroxypropionate/4-hydroxybutyrate (HP/HB) cycle [56], the dicarboxylate/4-hydroxybutyrate (DC/HB) [57] cycle, and the reductive glycine pathway [42]. Extensive reviews and comparisons of these pathways can be found elsewhere.

The CBB cycle, employed by photoautotrophs and some chemoautotrophs, is the most prevalent pathway for CO₂ fixation. However, chemoautotrophs face challenges due to complex nutrient requirements, process demands, and genetic manipulation difficulties, limiting their use in industrial applications [58]. Phototrophic microorganisms such as cyanobacteria and eukaryotic microalgae are more amenable to cultivation and have received attention in biotechnology. Nonetheless, scaling up production is hindered by technical challenges in photobioreactors, including light availability, distribution, and wavelength optimization [59].

Acetogens, on the other hand, offer an alternative for C1-based production. These microorganisms can convert CO or CO₂ into acetyl-CoA via the Wood-Ljungdahl pathway, using hydrogen as an energy source. Some acetogens also naturally produce ethanol or acetic acid and are used in syngas fermentations for biofuel and chemical production. Anaerobic gas fermentation by acetogens can convert waste streams and C1 gaseous substrates, but the product spectrum is limited by their metabolism. While genetic tools have been developed for well-known acetogens, genome editing remains challenging [60].

5. Methane

Methane is a highly potent greenhouse gas. A number of human activities lead to methane emissions, including oil, gas and coal production, agriculture (mainly animal husbandry) and landfills. Capture and bioconversion of methane emissions would be highly desired, however technically challenging for decentral emitters. Likewise, the gaseous substrate and its low solubility pose technical challenges for large scale bioreactor cultivation.

Methanotrophs have gained popularity for their ability to convert methane into biodiesel and chemicals [61,62]. They can be aerobic or anaerobic, including species from both the proteobacteria phylum and Verrucomicrobia, and some members of the Archaea domain. Industrial-scale manufacturing faces challenges due to low mass transfer rates and limited methane solubility in aqueous phases, requiring engineering solutions in reactor design and mass transfer enhancement.

The initial steps of methane assimilation are the oxidation to methanol and further to formaldehyde, from where assimilation and dissimilation follow the paths of methanol utilization in methylotrophs (mainly the RuMP or serine cycle). Most methanotrophs are aerobic bacteria, utilizing a methane monooxygenase to transfer electrons from methane to oxygen, consuming one NADPH per reaction. Anaerobic methane oxidation employs denitrification, sulfate or iron reduction. The C–H bonds of methane are highly inert, which explains why their activation for oxidation to methanol requires the investment of NADPH, despite being an oxidative reaction. Therefore, the aerobic utilization of methane cannot utilize the full energy content of methane but starts, from a redox viewpoint, at the degree of reduction of formaldehyde. Products made with methanotrophs include bioplastics (polyhydroxyalkanoates), microbial biomass for feed (and human food) use, and ectoine, a natural osmolyte of halophilic bacteria produced by some methanotrophs, which may find use in medicine, cosmetics, dermatology and nutrition [63].

6. Syngas

Syngas is a technical product of incomplete oxidation of hydrocarbons or biomass, yielding mainly carbon monoxide (CO) and hydrogen, with methane and CO₂ as by-products. It is especially an interesting substrate for acetogens which assimilate CO₂ and CO, with H₂ as the energy source. As for other gaseous substrates, dissolving the substrates in the liquid phase of bioreactors is a technical challenge.

Acetogenic bacteria are anaerobes using the Wood-Ljungdahl pathway to assimilate CO₂ and/or CO to acetyl-CoA, whereby hydrogen serves as energy source. One branch of the WL pathway uses THF as a carrier for a methylene group which is coupled with a molecule of CO to CoA by the multienzyme CO dehydrogenase/acetyl-CoA synthase. The product spectrum of acetogens is somewhat limited, being mainly acetate or ethanol. Metabolic engineering has enabled the production of a broader range of chemicals, like e.g. acetone and isopropanol, that were recently brought to pilot scale by Lanza Tech [64]. Another concept of CO fermentation with acetogens builds on two stage processes where the produced acetate is further converted to higher value products by other microbial strains [26, 65–67].

7. Synthetic C1 assimilation pathways

Recent research has focused on designing novel synthetic pathways for faster kinetics using advances in synthetic biology, bioinformatics, and biochemistry. These pathways have been discussed in detail in various reviews before [68–71]. They have been introduced into traditional industrial platforms through the incorporation of specific metabolic modules. Here we will discuss the challenges of the integration of C1 assimilation pathways into biotechnological chassis strains. Selected examples of chemicals produced with native and synthetic C1 assimilation pathways are summarized in Table 1.

Table 1
Some selected processes using C1 carbon sources for the production of chemicals.

Substrate	Organism	Product	Pathway	Titer	Yield or productivity	Reference
CO ₂	<i>Synechocystis</i> sp. PCC6803	d-Lactic acid	CBB	0.344 g/L		[91]
CO ₂	<i>Cupriavidus necator</i> H16	(R)-1,3-butanediol	CBB	2.97 g/L		[92]
CO ₂	<i>Komagataella phaffii</i>	Itaconic acid	CBB	2 g/L	2.7 mg/(g DCW h)	[77]
CO ₂	<i>Komagataella phaffii</i>	Lactic acid	CBB	0.4 mg/L	1.5 mg/(g DCW h)	[77]
CO ₂	<i>Citrobacter</i> BD11	Succinic acid	rTCA	15.02 g/L	0.313 g/(L h)	[93]
CO ₂	<i>Acetobacterium woodii</i>	Acetate	Wood Ljungdahl	51 g/L	0.56 g/(L h)	[94]
CO ₂	<i>Acetobacterium woodii</i>	Lactic acid	Wood Ljungdahl	1.7 g/L		[95]
CO ₂	<i>Eubacterium limosum</i>	Acetate	Wood Ljungdahl	5.02 g/L	0.03 g/(L h)	[96]
CO ₂	<i>Eubacterium limosum</i>	Butyrate	Wood Ljungdahl	0.145 g/L	0.001 g/(L h)	[96]
CO	<i>Clostridium acetivum</i>	Acetate	Wood Ljungdahl	18.0 g/L	0.036 g/(L h)	[97]
Formate	<i>Escherichia coli</i>	Lactic acid	rGP	0.108 g/L		[48]
Formate	<i>Cupriavidus necator</i>	Crotonate	CBB	0.148 g/L	0.0012 g crotonate/g formate	[98]
Formate	<i>Methylobacterium chloromethanicum</i>	Polyhydroxy butyrate (PHB)	Serine cycle	1.72 g/L	0.027 g PHB/g formate	[99]
Methane	<i>Methylobacterium buryatense</i> 5GB1	Lactic acid	RuMP	0.5 g/L		[100]
Methanol	<i>Methylophilus methylotrophus</i>	l-Lysine	RuMP	11.3 g/L		[101]
Methanol	<i>Komagataella phaffii</i>	3-Hydroxypropionic acid (3HP)	XuMP	48.2 g/L	0.23 g 3HP/g methanol	[102]
Methanol	<i>Komagataella phaffii</i>	Malate	XuMP	2.79 g/L		[103]
Methanol	<i>Komagataella phaffii</i>	free fatty acids	XuMP	23.4 g/L	0.078 g/g methanol	[104]
Methanol	<i>Ogataea polymorpha</i>	free fatty acids	XuMP	15.9 g/L	0.12 g/g methanol	[105]
Syngas	<i>Clostridium autoethanogenum</i>	Acetone	Wood Ljungdahl	5.8 g/L	3 g/(L h)	[64]
Syngas	<i>Clostridium ljungdahlii</i>	Isopropanol	Wood Ljungdahl	13.4 g/L	0.09 g/(L h)	[106]
Syngas	<i>Eubacterium limosum</i>	Acetate	Wood Ljungdahl	3.87 g/L	0.02 g/(L h)	[96]
Syngas	<i>Eubacterium limosum</i>	Butyrate	Wood Ljungdahl	0.899 g/L	0.005 g/(L h)	[96]

Synthetic methylotrophy has been achieved in various organisms, and heterotrophic microbes like *E. coli* and *K. phaffii* have been engineered to assimilate CO₂, making them synthetic autotrophs [26, 72–74, 75,76]. However, synthetic strains still lag behind natural producers in terms of metabolite yields and require further metabolic engineering [77]. The initial step in establishing a C1-based bioprocess involves selecting or designing a suitable microbial pathway for single carbon assimilation. This choice depends on the desired product and suitable microbial platforms to produce them. Autotrophic microorganisms provide the advantage to thrive directly on CO₂ and an energy source derived from light or chemical reactions. The CBB cycle, employed by photoautotrophs and some chemoautotrophs, is the most widespread CO₂ fixation pathway. Natural chemoautotrophs face challenges such as complex nutrient requirements and process demands, and difficulties in their genetic manipulation, so that their industrial applications are limited. Phototrophic microorganisms such as cyanobacteria and eukaryotic microalgae are more amenable to cultivation and have received attention in biotechnology [78,79]. Nonetheless, scaling up production is hindered by technical challenges in photobioreactors, including light availability, distribution, and wavelength optimization [80,81].

Methanol and formate assimilating microorganisms are the alternatives to autotrophs. Their use of liquid, highly water soluble, reduced C1 substrates make them independent from an additional energy source. Among them, methylotrophic yeasts are best characterized and well established for their metabolic engineering and large scale industrial fermentation. Methylotrophic bacteria are much less established which explains the interest to convert well-known hosts like *E. coli* into methylotrophic platforms. Despite exciting research results, formatotrophs are still in an early stage concerning their potential industrial application.

Acetogenic bacteria can assimilate CO and/or CO₂, using hydrogen as an energy source. Their product spectrum is limited (mostly to acetate or ethanol) and genome editing remains a challenge.

Overall pathway efficiency depends on pathway stoichiometry and thermodynamics. The demand of ATP and reduction equivalents (often expressed as NADH equivalents) are equally relevant as thermodynamically limiting reactions in a pathway which may have a delta Gibbs free energy close to zero. These thermodynamically limiting reactions are identified and quantified by pathway analysis with eQuilibrator [82] and expressed as max-min driving force (MDF), the minimal metabolic driving force calculated by adjustment of metabolite concentrations by

linear optimization to make all pathway reactions as favorable as possible. MDF is the inverse Gibbs free energy ($-\Delta_rG'$) of the reaction with the lowest numerical value.

The CBB cycle demands 3 mol of ATP and 2 mol of NADPH, with a moderate MDF of 2.34 kJ per mole of carbon assimilated. The rTCA and Wood-Ljungdahl pathways require less ATP and reducing power, but their low MDF values necessitate higher CO₂ concentrations for thermodynamic feasibility. For more reduced C1 sources like formate and methanol, four pathways exist: the XuMP, RuMP, rGlycine, and serine cycles. Among these, the serine cycle has the highest MDF per C-mol but requires substantial ATP and NADH. The XuMP cycle follows closely in MDF value, needing only one ATP per C-mol. Comparing the transaldolase (TA) and sedoheptulose-1,7-bisphosphatase (SBP) variants of the RuMP cycle reveals significant differences in MDF and ATP requirements, demonstrating the impact of enzyme selection on pathway thermodynamics. The Wood-Ljungdahl and rTCA cycles have the lowest MDF values, ranging between 0.53 and 0.67 kJ per C-mol, but require increased CO₂ concentrations.

8. Conclusion and outlook

Use of C1 carbon sources in industrial applications bears a lot of potential towards sustainable and carbon neutral or negative bioprocesses. However, each process should be designed considering its own requirements and specifications. Gaseous substrates such as CO₂, methane or syngas can be used for the production of renewable chemicals, as well as in more futuristic applications like CO₂ fixation in a Martian atmosphere [83]. However, mass transfer limitations in gas fermentations remain as an obstacle to solve, although several strategies are proposed [84]. Liquid C1 sources like methanol or formate are easier to use in large scale bioprocesses either with native or synthetic microorganisms. Methane and syngas are way more abundantly available, however to a large extent from fossil resources. The renewable production of methanol and formate [85–87], as well as syngas [88] is on the rise, but still need to be massively upscaled to serve as feedstocks for a C1 biotechnology that has a relevant impact on the global carbon balance [89] (Fig. 2).

So far, electricity demand for the reduction of CO₂ for future applications and scaling-up of bioprocesses with high productivities are the main challenges of sustainable C1-based productions to compete with conventional production cycles. Current synthetic strains are not yet efficient enough for large-scale industrial use and require further

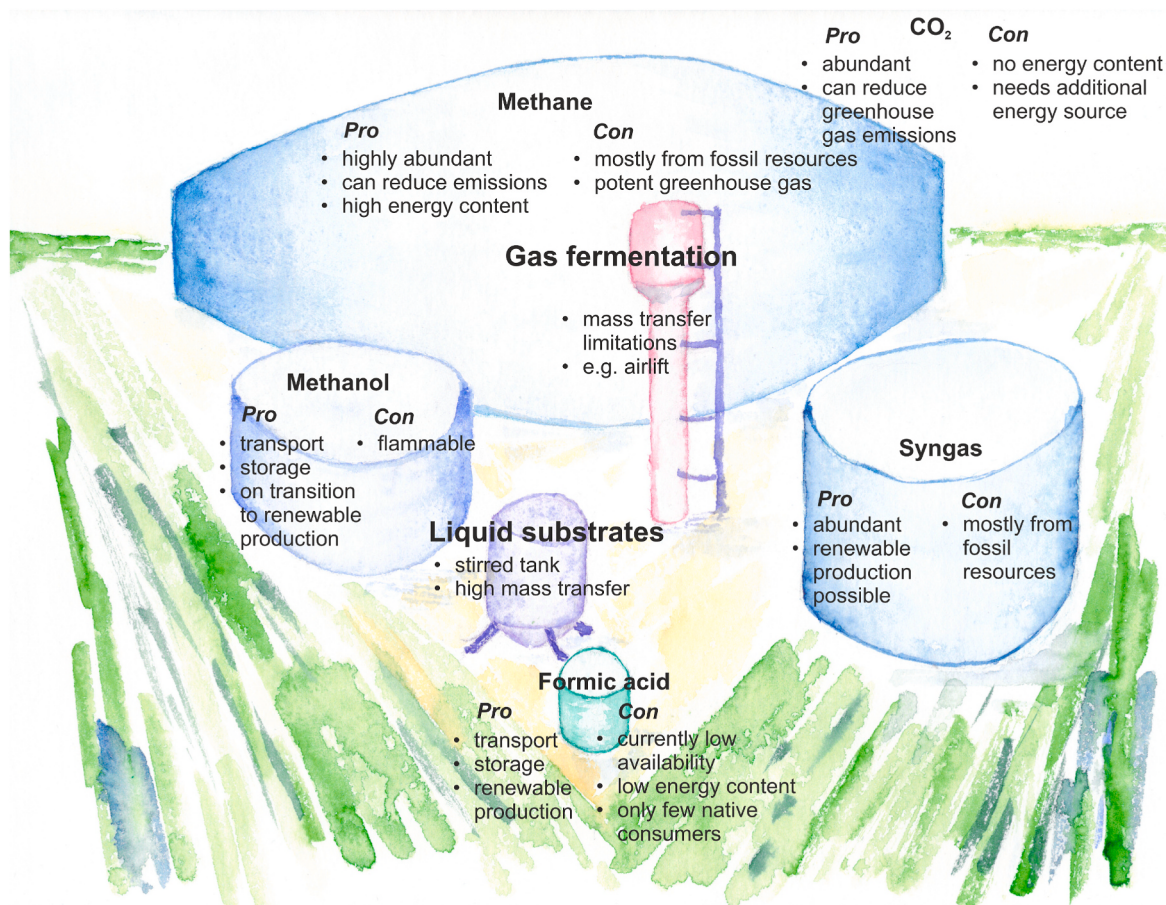


Fig. 2. Single carbon substrates for biotechnology. The main gaseous and liquid single carbon substrates and their advantages and disadvantages are highlighted. Tank sizes illustrate the respective relative annual production to date.

metabolic engineering, although some promising initial results have been published [48,77]. Expanding the substrate utilization range and integrating new synthetic pathways including their thermodynamic and kinetic models may help to facilitate the engineering of more efficient pathways with high MDF and low energy requirements for less thermodynamically favorable reactions. Electrochemical reduction of CO₂ into methanol or formate as an electron or carbon source is a promising approach, even though these processes are not yet competitive with fossil resources and more study is required especially focusing on the scale-up. However, as faradaic efficiencies for the conversion of CO₂ into formate is higher than methanol, formate use and formatotrophy might gain more attention in the future [90].

Gaseous C1 substrates (CO₂ and CO) are abundant and their bio-conversion into durable products would be among potential solutions to reduce greenhouse gas concentrations in the atmosphere. CO₂ and CO are main fractions of synthesis gas (together with H₂), and their fermentation bears great potential for environmentally beneficial production. Fermentation of gaseous substrates has some critical limitations however, first of all low mass transfer rates from the gas phase to the liquid fermentation broth, limiting volumetric productivities [84]. Liquid C1 substrates (methanol, formate) are favored by high water miscibility, which enables fast transfer to the fermentation broth, so that productivities are not limited by substrate supply. Beyond that, the ease of storage and transport of liquid substrates are major advantages over gaseous feedstocks.

While the production of chemicals from C1 substrates is widely researched (Table 1), only a limited number has already reached pilot or production stage. We conclude from these cases that processes using the WL pathway (which leads to acetyl-CoA) are mainly established for

production of C2 molecules like acetate and ethanol, or to other derivatives of acetyl-CoA like acetone or butyrate. Among other C1 pathways no clear product pattern can be identified which may be a sign for higher versatility of processes using these pathways.

The feasibility of an individual process is determined by several parameters, productivity being an important one. Others, such as achievable products and aeration demand need to be considered as well, making the best substrate and process for a given product a multi-parameter decision.

Authors' contributions

M.B., O.A. and D.M. have contributed equally to this work.

Declaration of competing interest

D.M. holds shares of FermX GmbH which develops cell factories for sustainable production of food and feed ingredients from C1 substrates. M.B. and O.A. declare no competing interests.

Acknowledgements

The work of M.B., O.A. and D.M. was supported by the Federal Ministry for Digital and Economic Affairs, the Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology, the Styrian Business Promotion Agency SFG, the Standortagentur Tirol, the Government of Lower Austria and ZIT - Technology Agency of the City of Vienna through the COMET Funding Program managed by FFG. O.A., and D.M. are members of the VIVALDI project

which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000441. The authors thank the Austrian Science Fund for support to M.B. and D.M. (FWF W1224, Doctoral Program on Biomolecular Technology of Proteins (BioToP)), to O.A. (Grant-DOI 10.55776/M2891) and to D.M. and O.A. (Grant-DOI 10.55776/I6795).

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