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Overcoming the energetic limitations of syngas fermentation

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The fermentation of synthesis gas (including carbon monoxide, carbon dioxide, and hydrogen) with anaerobic acetogens is an established biotechnological process that has recently been transferred to a commercial scale. The natural product spectrum of acetogens is natively restricted to acetate, ethanol, and 2,3-butanediol but is rapidly expanding to heterologous products. Syngas fermentation can achieve high carbon-efficiencies; however, the underlying metabolism is operating at a thermodynamic limit. This necessitates special enzymatic properties for energy conservation by acetogens. Therefore, the availability of cellular energy is considered to restrain the efficient production of energy-intense products with complex production pathways. The optimization of the feed-gas composition and other process parameters, genetic engineering, and integration with other biotechnologies is required to overcome this limitation.

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Introduction

Besides the need for alternative production routes for fuels and platform chemicals from renewable resources, technologies that enable mitigation of green-house gases are pertinent for a sustainable future [1]. The fermentation of reduced gases (i.e., carbon monoxide [CO] and hydrogen [H_2]), together with carbon dioxide (CO_2) is known as syn(thesis)gas fermentation. This platform technology has already proven to have enormous potential to contribute to a future bio-economy [2,3]. The utilization of syngas fermentation can off-set the use of fossil sources by replacing traditional production routes for fuels and chemicals [4]. In the long run, the process can contribute to a bio-economy that is based on direct recycling of CO_2, and might become intrinsically carbon-neutral. This is also because the source of the gases is flexible. Syngas can be generated from the gasification of biomass or municipal waste, and from renewable natural gas (mainly methane [CH_4]) by steam-reforming [2]. The renewable natural gas has to come from a source other than fossil natural gas to contribute to a sustainable bio-economy. For reviews on the production of renewable natural gas with Power-to-Gas technologies, the reader is referred to recent reviews on this topic [5,6]. Also, CO is the main component of certain gas streams from heavy industries [7].

The utilized microbial catalysts, which microbiologists refer to as acetogens, mainly produce acetate as their natural fermentation product [8]. The conditions can be steered toward the production of alternative natural products such as ethanol, and 2,3-butanediol [9,10]. Furthermore, progress on the implementation of molecular biology tools and genetic engineering strategies now allows to broaden the product spectrum to non-natural fermentation products or to optimize selective production of natural products [11••,12,13••].

This review briefly summarizes the knowledge on the variety of acetogens capable of syngas fermentation, the achieved product spectrum, and new insight into the underlying physiology. The focus, however, is on the most recent achievements for overcoming the energetic limitations of syngas fermentation by metabolic modeling, genetic engineering, and integration with other bioprocessing technologies.

Open culture-based versus pure culture-based production platforms

For the implementation of a syngas fermentation process either open cultures or pure cultures can be considered [14]. For open cultures, process parameters can be optimized to establish a microbiome that potentially generates a product of interest. The spectrum of possible products is limited to naturally occurring products, since genetically engineered strains cannot be specifically maintained in an open culture. However, the products may result from a combination of metabolic pathways available in different microbes. Thus far, the achieved volumetric production rates and titers have been low in

open cultures [15–17], and it is not known whether they can be improved to industrially relevant rates and titers. Higher production rates and titers have been reported with pure cultures [14,18–21]. Also the product spectrum can be extended by genetic engineering [2]. Important bacteria capable of syngas fermentation are discussed in the next section.

The spectrum of acetogens capable of syngas fermentation

Most commonly used acetogens for syngas fermentation are bacteria in the genus *Clostridium*, however, a few other species have also interesting properties (Table 1). Other recent reviews present more complete lists [2,10,22]. The products that naturally occur with syngas fermentation are mainly acetate, ethanol, and to a lesser extent 2,3-butanediol [23]. Other acetogens have a broader product spectrum and *Clostridium carboxidivorans*, for example, also naturally produces *n*-butanol and *n*-hexanol (Table 1) [18]. The increasing availability of genome sequences may result in the identification of more efficient production hosts, the capabilities of producing other interesting products, and strategies for genetic engineering [24–27].

The Wood-Ljungdahl pathway and the physiology of acetogens

Acetogens are capable to fix CO_2 via the Wood-Ljungdahl pathway (WLP) [8,28,29]. This linear process is considered the oldest carbon-fixation pathway [30]. It results in acetyl-CoA, which is further utilized for biomass growth and acetate production. While the production of one mole of acetate results in the formation of one mole of ATP by substrate-level phosphorylation, the pathway does not generate net ATP because it also utilizes one mole of ATP for the activation of formate to formyl-tetrahydrofolate (Figure 1) [31,32]. For further cellular energy-conservation in the form of ATP for biomass growth and cellular maintenance processes, some acetogens, such as *Moorella thermoacetica*, contain an energy-converting hydrogenase (Ech), which generates a membrane potential [33,34]. *M. thermoacetica* also contains cytochromes and menaquinone while the exact mechanism of energy conservation (i.e., the generation of a membrane potential to drive ATP synthase) in this acetogen has not been elucidated (Figure 1) [32]. These acetogens typically are not efficient alcohol producers from syngas [35]. Researchers only discovered a few years ago how acetogens without cytochromes can generate additional cellular energy for

Table 1

Important microbes capable of syngas fermentation

Organism	Rnf/ATP synthetase (H^+/Na^+)	AOR	Gaseous Substrates	Products	Interesting features	References
Acetobacterium woodii	Na^+	No	H_2/CO_2	Acetate; ethanol only when fermenting sugars	Highest reported acetate production, genetic tools	[19,37,76–78]
Butyribacterium methylotrophicum	coupling ion not determined			nd[a]	$H_2/CO_2/CO$	Acetate, *n*-butyrate, ethanol, *n*-butanol
n-Butanol production	[26,79]					
Clostridium autoethanogenum	H^+	Yes	$H_2/CO_2/CO$	Acetate, ethanol, 2,3-butanediol, lactate	Industrially relevant, genetic tools, CRISPR/Cas9 reported, GEM[b]	[12,56,80,81]
Clostridium carboxidivorans	H^+	Yes	$H_2/CO_2/CO$	Acetate, *n*-butyrate, ethanol, *n*-butanol, *n*-caproate, *n*-hexanol	Hexanol-Butanol-Ethanol fermentation	[82–84]
Clostridium ljungdahlii	H^+	Yes	$H_2/CO_2/CO$	Acetate, ethanol, 2,3-butanediol, lactate	Genetic tools, CRISPR/Cas9 reported, widely used model organism, GEM[b]	[13••,24,55,85,86]
Eubacterium limosum	Na^+	nd	$H_2/CO_2/CO$	Acetate, *n*-butyrate	*n*-Butyrate production	[87,88]
Moorella thermoacetica	H^+ (Ech and cytochromes instead of Rnf)	No	H_2/CO_2	Acetate	Thermophilic, contains cytochromes and menaquinone, GEM[b]	[34,89,90]
Sporomusa ovata	H^+ (Ech and cytochromes instead of Rnf)	Yes	H_2/CO_2	Acetate, Ethanol	Used to study microbial electrosynthesis, contains cytochromes and ubiquinone	[91–93]

[a] nd, not determined.
[b] GEM, genome-scale metabolic model.