C_1-gas (syngas, CO_2) bioconversion to biofuels and bioproducts

Christian KENNES and María C. VEIGA

University of La Coruña, Chemical Engineering Laboratory, Faculty of Sciences and Center for Advanced Scientific Research/Centro de Investigaciones Científicas Avanzadas (CICA), BIOENGIN group, E – 15008 – La Coruña, Spain
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Presenting author email: Kennes@udc.es

Introduction

Increased attention has been paid recently to issues related to the environmental effects of greenhouse gases and other air pollutants, such as CO_2 and also CO, which are both emitted during combustion processes, but also from other industrial activities. Similarly to this, syngas can be obtained from the gasification of different carbonaceous materials, including biomass and wastes, and does also contain CO and often CO_2, besides hydrogen. Technologies have recently been developed, and are nowadays being optimized, for the conversion and valorization of such gases, yielding, whenever possible, valuable compounds, and following that way a sustainable circular economy approach.

In that sense, it is known that acetogenic bacteria grow naturally on C_1 gases (e.g., CO_2, CO, syngas), under anaerobic conditions, producing short chain carboxylic acids, mainly acetic acid (two-carbon, C_2, compound) and, occasionally some longer carbon chains, such as butyric (C_4) or caproic (C_6) acids. Some of those bacteria can also produce high concentrations of alcohols as end metabolites, e.g., ethanol (C_2) mainly, and, sometimes butanol (C_4) or lower amounts of hexanol (C_6), although this is less common and limited to few species only, such as *Clostridium ljungdhalii* (Mohammadi et al., 2012), *Clostridium autoethanogneum* (Abubackar et al., 2015), *Clostridium carboxidivorans* (Fernández-Naveira et al., 2019), or *Clostridium aceticum* (Arslan et al., 2021), among others; following, in all cases, the so-called Wood-Ljungdhal pathway (Kennes-Veiga et al., 2023).

Although carboxylic acids and ethanol are valuable commercial products, it is necessary to further broaden the range of compounds that can be obtained from anaerobic C_1 gas fermentation, to other alternative biofuels or bioproducts, which has been the goal of our research over the past few years and is addressed hereafter.

Experimental procedures

Most of the assays on acetogenic fermentation have been performed in fully automated anaerobic stirred tank BIOFLO120 bioreactors (Eppendorf, Juelich, Germany). The other, below described, fermentation processes have either been performed in the same BIOFLO reactors, operated either under aerobic or anaerobic conditions, depending on the specific process, or in home-made customized bioreactors. All pure or mixed microbial cultures used in this research, as well as the employed materials and analytical methods have recently been described in the literature and details can be found in the reference list provided at the end of this Abstract, for further detailled information.

Results and Discussion

In order to broaden the range of metabolites obtained from C_1 gases, two different approaches were adopted in our studies and will briefly be described; on one side the engineering of acetogenic bacteria and on the other side the set-up of two stage bioconversions, as explained hereafter.

Two-stage bioreactors: Since acetogenic bacteria efficiently produce short chain carboxylic acids and sometimes ethanol, it appeared to be feasible to produce one or several of those compounds, depending on the needs, from C_1 gases, in a first stage (i.e., first bioreactor), followed by a second stage (i.e., second bioreactor) focusing on the subsequent bioconversion of metabolites from that first stage.

In that sense, since some aerobic bacteria are able to accumulate biopolymers (e.g., PHA, PHB) from short chain fatty acids, our results demonstrated that carboxylic acids (C_2, C_4 and/or C_6) produced from C_1 gases in a first, anaerobic, bioreactor, can subsequently be fed to a second sequencing batch, aerobic, bioreactor, in which the acids are converted into biopolymers (Portela-Grandio et al., 2021).

Another alternative consisted in producing medium chain carboxylic acids (MCCA), e.g., caproate (C_6) and/or caprylate (C_8), which have rather higher commercial values than short chain fatty acids such as acetic acid. This was possible by feeding a mixture of ethanol and acetic acid, produced from C_1 gases by acetogenic bacteria, to a second stage, in which other anaerobic bacteria performing chain elongation through the reverse oxidation pathway converted these compounds, as electron donor and electron acceptor, into MCCA (Fernández-Blanco et al., 2022).

Finally, to provide an additional recent success story of such two-stage processes; in other assays, C_1 gases were converted into either acetic acid or a mixture of C_2, C_4, C_6 carboxylic acids. The latter were then fed to bioreactors containing oleaginous yeasts such as *Rhodosporidium toruloides* (Robles-Iglesias et al., 2021) or *Yarrowia lipolytica* (Naveira-Pazos et al., 2022), which allowed to accumulate high concentrations of lipids (microbial oils), suitable for the production of biofuels, e.g., biodiesel.

Engineered bacteria: The range of products that native acetogenic bacteria can produce through gas fermentation is limited, among others because of bioenergetic barriers. Production of compounds such as acetone or iso-propanol is thus not possible in such non-modified bacteria. However, as part of a collaborative European project, the acetone biosynthetic pathway was constructed by combining genes from *Clostridium acetobutylicum* and *Clostridium aceticum* in an engineered *Acetobacterium woodii* strain, allowing that organism to produce acetone directly from C_1 gases in bioreactors. Interestingly, that engineered strain was found also to be able to produce iso-propanol, another valuable commercial bioproduct (Arslan et al., 2022).

Conclusions

Acetogenic bacteria are suitable organisms for the production of either short chain carboxylic acids or alcohols ($C_2 – C_6$) directly from C_1 gases (CO_2, CO, syngas). It can be concluded that the range of valuable products that can be obtained from such process can successfully be broadened with engineered bacteria, producing compounds such as acetone or iso-propanol, among others. Alternatively, carboxylic acids and/or alcohols obtained from anaerobic C_1 gas fermentation can be used as substrates, in a second stage, by different aerobic or anaerobic bacteria or yeasts to produce additional interesting products such as biopolymers (bioplastics), medium chain carboxylic acids, or microbial oils.

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