Bioprocesses for waste, syngas and carbon dioxide valorisation

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Introduction

Environmental pollution due to the accumulation or poor management of solid waste, wastewaters or polluting gases is a major issue in modern societies, including climate change due to the emission of greenhouse gases such as CO_2 (Kennes, 2023). Solid waste, renewable biomass and other related feedstocks can be valorised and converted into syngas through gasification processes. The composition of syngas depends, among others, on parameters such as the gasification conditions, but are often composed of CO, CO_2, and H_2 as major products. Those three gases can be converted into acetic acid and, occasionally, ethanol, by acetogenic bacteria (Kennes-Veiga et al., 2023, 2024). Similarly, carbon dioxide, a well-known greenhouse gas and common industrial gas pollutant, can be metabolised by acetogens, in the presence of hydrogen, yielding the same end products, *i.e.*, acetate and/or ethanol.

The bioconversion of fatty acids, such as acetate, to added value commercial compounds, though the so-called carboxylate platform has been gaining significant interest in recent years. Those fatty acids can be obtained from the acetogenic fermentation of solid waste, biomass, wastewaters or gas emissions, as mentioned above. Short chain fatty acids are generally considered to be cheaper substrates than carbohydrates such as glucose, justifying the recent increasing interest in such carboxylic carbon sources.

Therefore, some of our present research interest, reported here, deals with the acetogenic anaerobic bioconversion of C_1-gases (*i.e.*, one carbon gases) (*e.g.*, CO, CO_2, found in industrial gas emissions and in syngas) into acetic acid and/or ethanol, for the subsequent bioconversion of the latter into high value compounds of commercial interest. In this study, special interest is given to the production of carotenoids together with microbial oils which can be generated by some specific oleaginous yeasts from acetate (Naveira-Pazos et al., 2023), as well as to the production of caproate, a high-value carboxylate, produced through chain elongation of acetate by bacteria such as *Clostridium kluyveri*, in the presence of ethanol (Fernández-Blanco et al., 2022), or *Megasphaera hexanoica*, in the presence of lactate (Wang et al., 2024a).

Experimental procedures

The bacterial cultures described in the present studies were obtained from DSMZ (Germany), while the engineered *Yarrowia lypolytica* yeast strain was constructed as described elsewhere (Robles-Iglesias et al., 2023). The anaerobic and aerobic fermentations were performed either in automated stirred tank BIOFLO120 bioreactors (Eppendorf, Juelich, Germany) or in home-made automated bioreactors. Additional information on operating conditions and analytical methods can be found in the references cited at the end of this Abstract.

Results and Discussion

In our research, in order to broaden the range of valuable products that can be obtained from C_1-gases, different compounds were obtained through the carboxylate platform. As two illustrative examples of our studies, data are presented here on the production of carotenoids, used as nutraceutical compounds, and the production of caproic acid, an organic acid, with different industrial applications, and higher commercial value than short chain fatty acids, such as acetate.

Carotenoids: Carotenoids can be obtained from the fermentation of sugars (*e.g.*, glucose) by yeasts and also from acetate, considered, more recently, as a more attractive and cheap substrate. In the present study, either syngas or (CO_2 + H_2) were continuously fed to an anaerobic bioreactor, inoculated with an acetogenic strain, for the production of acetate as attractive substrate, in a first stage. The acetate-rich medium was then subsequently fed to a second, aerobic, reactor, inoculated with an engineered *Yarrowia lypolytica* yeast strain accumulating carotenoids and microbial oils. Acetate concentrations up to about 30 g/L were achieved in the acetogenic bioreactor. The carboxylic acid was then subsequently fully metabolised by the *Yarrowia* strain yielding high carotene concentrations from such fatty acids substrates, compared to literature data, exceeding 1 g/L. Besides, the yeast was able to accumulate lipid concentrations of up to about 50%. The integrated acetogenic-yeast fermentation is thus an attractive process to produce carotenoids and microbial oils.

Caproic acid: While acetogenic bacteria, *e.g.*, *Clostridia* or species such as *Acetobacterium woodii*, can accumulate fatty acids, *e.g.*, acetate, other anaerobic bacteria can elongate such short chain fatty acids to produce medium chain carboxylic acids (*e.g.*, caproate) of higher commercial value. In that sense, *Megasphaera hexanoica* is able to use lactate, a waste by-product from industries such as the dairy industry, as electron donor, in the presence of acetate, as electron acceptor. Therefore, a co-culture of *A. woodii* and *M. hexanoica* was fed a mixture of lactate and CO_2, in either presence or absence of iron generating hydrogen through chemical reaction. In the co-culture, (CO_2+H_2) are first converted to acetate, which is then metabolised by *M. hexanoica* together with lactate, to produce caproate and additional H_2; the latter being used by the acetogenic strain to produce more acetate needed by the *Megasphaera* spp. for further chain elongation. The presence of iron in the medium also produces additional H_2, boosting caproate production (Wang et al., 2024b). This allows to successfully produce caproate from CO_2 and lactate.

Conclusions

Carbon dioxide and syngas can efficiently be valorised through their bioconversion, by acetogenic bacteria, into acetate, followed by a second stage bioconversion process of such acetate into higher value end products, through the carboxylate platform. In that sense, acetate was proven to be efficiently converted to carotenoids and lipids by engineered oleaginous yeasts. Besides, chain elongation of acetate, in the presence of dairy waste products such as lactate, yielded high value caproate. These results offer and interesting biorefinery approach to valorise industrial waste products as well as carbon dioxide or syngas, generating high value end products of commercial interest.

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