Valorisation of organic waste for the production of high-value molecules

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Introduction

Biomethanation has become a frequently used method to treat organic waste, leading to the production of biogas and solid/liquid waste called digestate. The biogas contains around 50% methane and 50% CO_2, while the digestate is made up of high levels of ammonium and volatile fatty acids (VFA). Digestate is currently used as a fertiliser due to its high nitrogen content (Manu et al., 2021; Okoro-Shekwaga et al., 2021). However, an incomplete transformation of the organic waste into biogas increases the VFA contents, which can be utilised by purple non-sulfur bacteria (PNSB). Through the versatile metabolism of these bacteria, these VFA could be valorised into single-cell proteins, vitamin B12, bioplastics in the form of polyhydroxyalkanoates (PHA), carotenoids or hydrogen (Alloul et al., 2019; Bayon-Vicente et al., 2020; Cabecas Segura et al., 2022; Warren and Deery, 2009). Different studies have described the photoassimilation of acetate, butyrate and valerate, but also combinations of VFA in laboratory settings, but also using real wastewater (Alloul et al., 2019; Bayon-Vicente et al., 2020; Cabecas Segura et al., 2022; De Meur et al., 2020; Leroy et al., 2015). The aim of this project is to use digestate derived from a small-scale biomethanation plant running on kitchen waste to produce carotenoids and PHA.

Materials and Methods

To assess the assimilation of VFA by purple bacteria, *Rhodospirillum rubrum* S1H ATCC 25903, as well as co-cultures of the latter with *Rhodobacter capsulatus* DSM 1710 and *Cereibacter sphaeroides* ATCC 11167 (at equal OD_{680} values) were grown anaerobically in MELiSSA medium containing a blend of 40% acetate, 20% propionate, 15% butyrate, 5% isobutyrate, 10% valerate and 10% isovalerate as carbon sources (synthetic digestate) at a light intensity of 120 µmol m^{-2} s^{-1} (Halogen lamps of 10W, 100 lumens, 3000K or LED light strips at 525 nm, 592 nm and 850 nm) and on a shaking plate at 180 rpm. In addition, the concentration of NH_4Cl in the synthetic digestate medium was increased two- to tenfold to determine the effects of high ammonium levels on the bacterial strains. Naïve cultures only grew in medium containing 35 mM NH_4Cl, while acclimated cultures were grown in increasing levels of NH_4Cl (35 mM, 70 mM, 140 mM, 350 mM). The organic acid assimilation was analysed using an F5 column on a LC/MS-MS (AB Sciex ZenoTOF® 7600 System), while the ammonium content was assessed using a fluorometric assay (Holmes et al., 1999). The proportions of the bacterial strains were analysed using the plate-count method (CFU/mL), 16S rDNA sequencing and data-dependent proteomic analysis (DDA).

Results and discussion

In a first phase, the growth of *Rs. rubrum* and co-cultures of *Rs. rubrum, Rh. capsulatus* and *C. sphaeroides* in the synthetic digestate with regular levels of ammonium was assessed. The bacterial cultures were all able to grow to the expected levels and were able to assimilate the different VFA in the classical MELiSSA medium. Cultures of *Rs. rubrum* assimilated acetate and propionate rapidly, followed by (iso)butanoate and valerate, while isovaleric acid was assimilated last (Figure 1A).

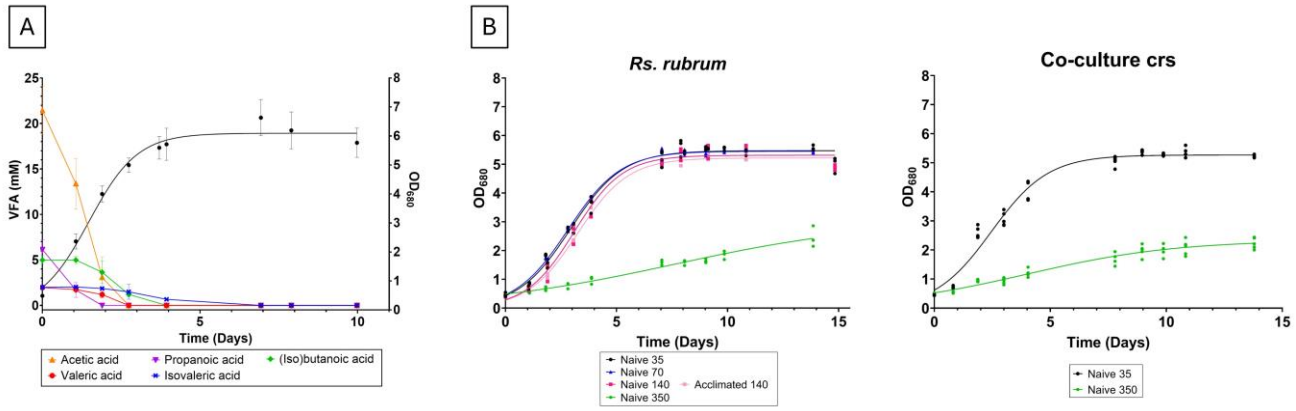

Figure 1. (A) Growth of *Rhodospirillum rubrum* (OD_{680}) (right y-axis) and assimilation of the volatile fatty acids (VFA) in the synthetic digestate (left y-axis) over time. Acetate was assimilated rapidly, while isovaleric acid was assimilated last. Mean ± SD, *n* = 5. (B) Growth of *Rhodospirillum rubrum* (left) or co-cultures (right) in different levels of ammonium. No growth inhibition could be observed in cultures growing in 35 mM, 70 mM or 140 mM. Bacterial cultures with 350 mM NH_4Cl did suffer from growth inhibition.

As digestate contains higher levels of ammonium (Manu et al., 2021), the resistance to ammonium of the mono-culture of *Rs. rubrum* and the co-cultures of the three strains was evaluated. Culture medium containing 70 mM or 140 mM of ammonium did not hinder the growth of *Rs. rubrum*, however, statistically significant reduction of bacterial growth could be observed in culture medium containing 350 mM ammonium. A similar effect was observed in co-cultures of the three strains (Figure 1B). Although the initial inoculants for the co-cultures contained a similar number of bacteria, it could be observed that *Rs. rubrum* became the most prominent strain by the stationary phase. This was confirmed by the plate-count method, 16S rDNA sequencing and DDA.

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