H_2/CO_2 addition during microbial batch fermentation of orange peel wastes for chain elongation of short chain carboxylates

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Oranges, with a global consumption exceeding 70 million tons annually, are renowned for their bioactive compounds such as vitamin C, flavonoids, phenolic acids, and essential oils (EO) (Mekouar, 2021). However, their use generates large quantities of orange peel waste (OPW), which, if not properly managed, can lead to the release of greenhouse gases emissions, soil and water contamination, pest attraction and unpleasant odors (Intergovernmental Panel on Climate Change, 2021). A promising solution to solve this environmental issue lies to microbial bioconversion of OPW to short- chain (SC) and medium- chain (MC) carboxylates. To propose an eco-friendly valorization alternative, we investigated the synthesis of medium-chain carboxylic acids through microbial fermentation of OPW under mild conditions. Although literature confirms the detrimental effect of limonene and other EO on CH_4 production during fermentation, there is no information about its effect on the production of carboxylic acids (CA) (Henry, G. et al., 2023). The aim of the present work was to test the influence of EO on the fermentation of OPW to alcohols, short and medium-chain carboxylic acids during acidogenic fermentation. In addition, we assessed the possibility to elongate the existing SC and MC carboxylates through the addition of H_2 and CO_2, as complementary sources of electron-donors and carbon feedstock respectively. This innovative approach would help to valorize polluting CO_2 in the production of higher-value products.

OPW was submitted to fermentation either untreated or after partial removal of EO. In a first approach, isopropanol was used as extractant to remove EO from OPW (chemical method). In another approach, the flavedo was removed with a knife thus reducing the EO concentration (physical method). The treated and untreated OPWs were ground in a miller until a paste was formed. OPW was introduced in 2 L glass reactors and mixed with 140 mL of distilled H_2O and 20 g of activated sludge (as inoculum), to reach a concentration of 70g_COD_OPW/ l_mixed_liquor. The headspace of each reactor was flushed with N_2 then pressurized at 1.6 bar absolute pressure. All reactors were orbitally shaked at 120 rpm at 35 °C in the dark. Every 2 days, samples were collected from the liquid to monitor CA concentration. The gas production was monitored with the total pressure and gas composition. The headspace pressure was adjusted after each sampling to 1.6 bar_abs. by releasing the excess fermentation gas or by supplying N_2. Each test was conducted in triplicate. For carboxylates elongation, the same fermentation protocol was employed but, N_2 was replaced by a H_2/CO_2 mixture in a ratio 3/1 mol/mol, until an absolute pressure of 1.3 bar was reached.

Limonene concentration in OPW was about 3.8 mg/g_fresh_matter (FM) while this value decreased down to 0.2 and 0.3 mg/gFM_OPW when chemical and physical removal methodologies were applied respectively. Although both procedures reduced limonene concentrations almost entirely, fermentation in both cases progressed quite differently. As compared to untreated OPW, the physical EO removal method led to similar CO_2, H_2, ethanol and CA productions, while the chemical removal method reduced significantly these productions (Fig. 1a-b). Valeric acid was better produced in the untreated OPW. All these results suggest that EO, including limonene, have no detrimental effect on acidogenic fermentation under these conditions.

As regards carboxylates chain elongation, untreated OPW were assessed in the presence of H_2/CO_2 mixture as well as N_2 atmosphere to compare. An increase of SC carboxylates concentration was observed during the first 10 days of batch fermentation (Fig. 2a) under N_2 atmosphere. By day 15, when the cumulative production of H_2 diminished the concentration of these SC carboxylates stabilized. At the same time, ethanol concentration decreased in favor of acetic acid concentration. On the other hand, in batch fermentation of OPW under H_2/CO_2 atmosphere a higher concentration in valeric acid was detected along with gases consumption. From day 25, valeric acid production started to decrease, coinciding with the formation of MC carboxylates, such as heptanoic and octanoic acids (Fig. 2b). These results suggest that our microbial community use H_2 as an electron donor, and CO_2 as a carbon source, to elongate SC carboxylate for the production of longer carboxylates than in the case of batch fermentation with N_2 atmosphere.

These results suggest that EO, including limonene, are not harmful for CA production by acidogenic fermentation. Moreover, untreated OPW along with CO_2/H_2 addition can be profited for MC carboxylates productions under mild conditions. Thereby, these results open new possibilities to valorize OPW without relying on costly chemical treatments, leading instead to the production of higher value molecules in the context of circular economy.

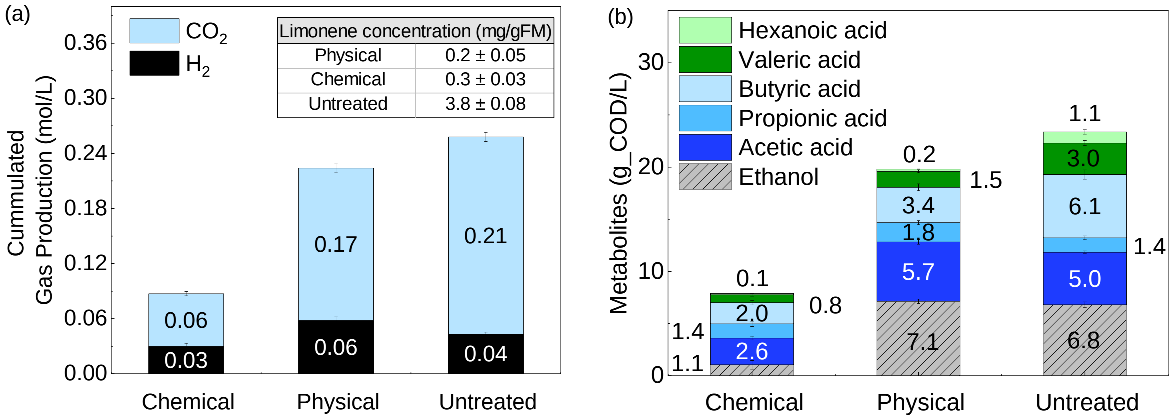

Figure 1. Results after 20 days of fermentation of untreated OPW and OPW treated to remove essential oils either by isopropanol extraction (Chemical treatment) or by peeling the flavedo layer (Physical treatment): a) cumulated gas production; b) metabolites production

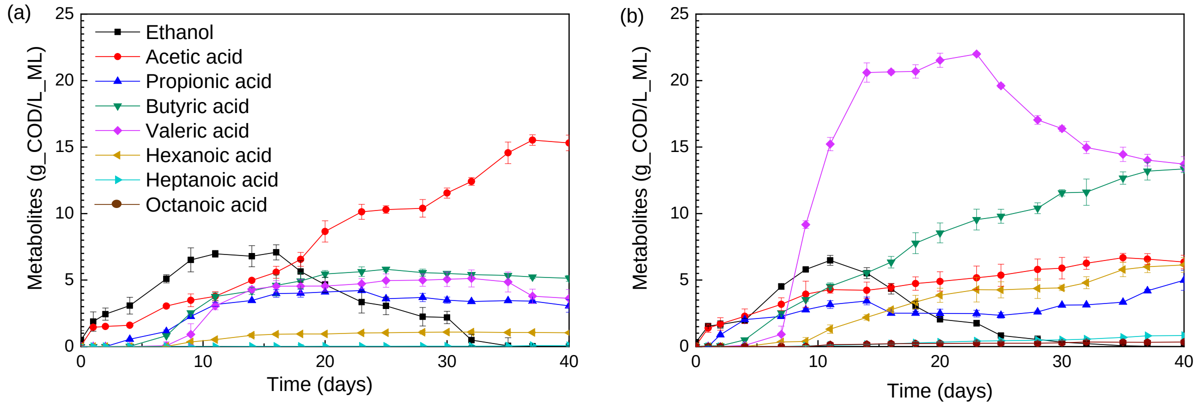

Figure 2. Evolution of metabolites concentrations in batch fermentation of orange peel waste for 40 days under: a) N_2; b) H_2/CO_2. Mean and standard deviation of triplicates.

References

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