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Biocatalytic performance of *Butyribacterium methylotrophicum* in the long-term conversion of synthesis gas produced from low-grade lignin gasification by *Butyribacterium methylotrophicum*

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Second-generation biorefineries produce large streams of low-grade lignin. Its thermochemical conversion, through gasification, enables the carbon recovery from an otherwise recalcitrant by-product. The main product of gasification is producer synthesis gas (PS), which is mainly composed by carbon monoxide (CO), carbon dioxide (CO_2), hydrogen (H_2), methane (CH_4) and minor impurities. Carboxydotrophic acetogenic bacteria can utilize CO and CO_2 as carbon and energy source, and convert them into biomass, biofuels and biochemicals through the Wood-Ljungdahl pathway.

This work focused on the sequential batch fermentation of PS by *Butyribacterium methylotrophicum*. The PS was produced by gasification of technical lignin from a 2nd generation ethanol biorefinery, and its major components were [%vol.]: 24.0 CO, 16.5 CO_2, 23.9 H_2, 13.6 CH_4 and 18.1 N_2. A two-phase bioconversion process was performed envisioning the production of butyric acid from the gaseous substrate. Firstly, cells were grown at pH 7.0 for biomass and acetate accumulation. After 232 hours, the pH was reduced to 6.0 and the culture medium was supplemented with acetic acid to promote butyrate production. The supply of PS to the bioreactor was performed on demand for a total of 380 hours.

B. methylotrophicum required an initial adaptation period to PS, after which it maintained its viability and metabolic activity for the entirety of the process time. Overall, 1.8 NL of PS were introduced on demand in the bioreactor. Over the sequential batches, with each renewal of nutrient solution, there was a continuous reduction of the PS infeed rate, from 20.3, to 10.4, to 3.2, and finally, to 0.9 NmL/h, probably caused by accumulation of potentially inhibiting gaseous compounds in the bioreactor. CO_2 and H_2 varied between 5.7-9.2%$_{mol}$ and 1.5-7.1%$_{mol}$ inside the bioreactor headspace, respectively, whereas CO was never detected, indicating its full fixation by *B. methylotrophicum*. This resulted in a total carbon fixation of 94.6%$_{mol}$ of the CO+CO_2 supplied. Acetate was mostly produced during the first bioconversion phase, reaching a concentration of 51.1 mmol/L_{medium}, correspondent to a specific production yield of 11.9 mmol/NL_{used}/g_{CDW}. Butyrate was produced along the 380 hours, but the specific production yield started at 0.3 mmol/NL_{used}/g_{CDW} and reached 0.9 mmol/NL_{used}/g_{CDW} in the second conversion phase. These results show the potential of integrating gasification and PS bioconversion towards a carbon neutral and sustainable production of chemical building blocks and biofuel precursors.