

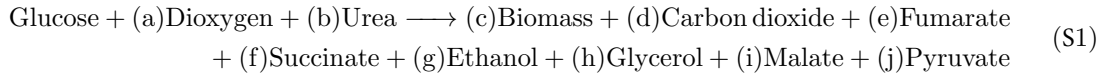
The effect of pH, metal ions, and insoluble solids on the production of fumarate and malate by *Rhizopus delemar* in the presence of CaCO_3

Supplementary Material

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1 Mass Balance

A black box model based on the metabolic map for *R. delemar* provided and discussed by (Naude & Nicol, 2017) was mathematically described through a mass balance over the system with the total chemical equation (Equation S1) below. Initial amounts of components from the growth run were accounted for using HPLC-determined time-zero sample concentrations in every production run. The biomass formula used was $\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2}$ (Nielsen & Villadsen, 1994), and tests were conducted where the initial and final biomass measurements were taken to quantify biomass growth during production.



Balances were done for C, N, and Degree of Reduction (DOR). In addition, all the HPLC determined values and biomass measurements were used to fully specify the system. The unknowns were predicted by solving the equation at every sampling point, and with data normalization, showing the carbon distribution over the cultivation period. The CO_2 determined from Equation 1 only represented carbon dioxide produced from the fermentation, and did not include the contribution from the dissolution of CaCO_3 .

2 Metabolite distribution of *R. delemar*

Figure S1, Figure S2, and Figure S3 below show the carbon distribution across the 20, 60 and 100 g L^{-1} fermentations. A steady increase in the CO_2 was observed across all fermentation conditions, an indication of increasing energy requirements towards the end of the fermentations. The increased energy demands could be as a result of cell maintenance (an energy intensive procedure), or a factor of increased transport costs.

Dicarboxylic acid excretion does not occur through diffusion, and therefore relies on transporters which require ATP (Swart, Le Roux, Naude, De Jongh, & Nicol, 2020). The energy cost of excreting dicarboxylic acids is directly influenced by the pH of the medium. From highest to lowest pH, the transporter selected changes as follows; the uniport, symport and antiport, respectively, with the lowest pH transporter incurring the largest energy cost (Taymaz-Nikerel et al., 2013).

Another factor that influences the acid excretion energy cost is the extracellular concentration. An increase in extracellular concentration (batch fermentations) results in increased energy requirements to overcome the resultant concentration gradients. Therefore, a contribution to the increased energy demands likely occurred as the extracellular concentrations increased due to dicarboxylic acids accumulation during the course of the fermentation.

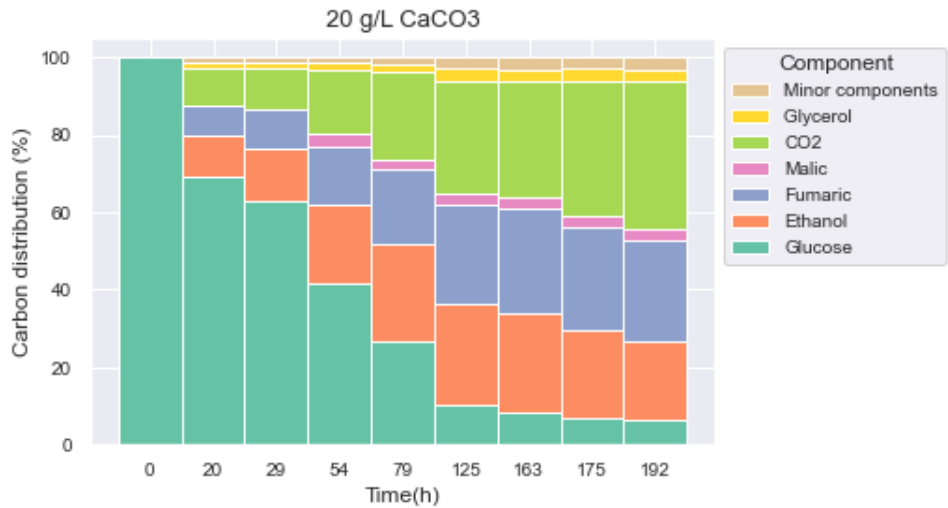


Figure S1. Metabolite distribution of *Rhizopus delemar* with the minor components including biomass, succinic acid and pyruvic acid. Cultures were incubated at 34 °C, 200 rpm in the presence of 20 g/L CaCO₃.

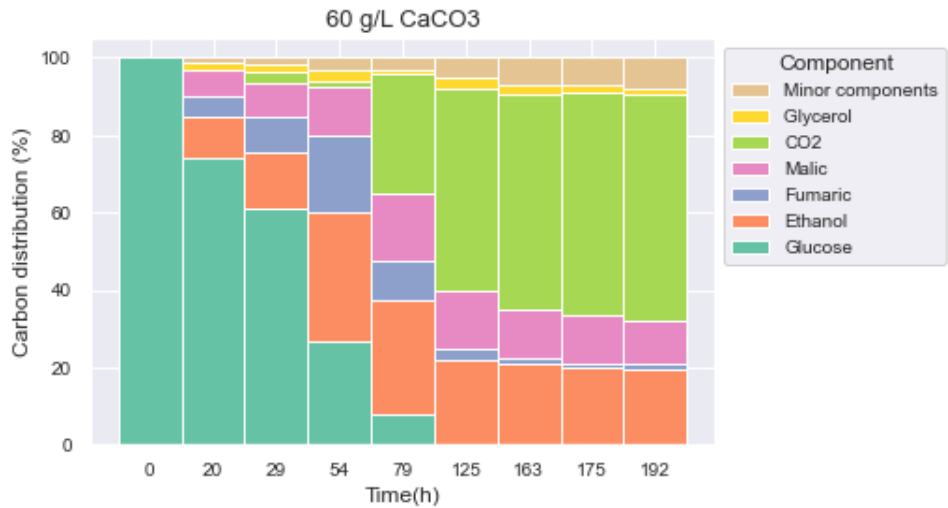


Figure S2. Metabolite distribution of *Rhizopus delemar* with the minor components including biomass, succinic acid and pyruvic acid. Cultures were incubated at 34 °C, 200 rpm in the presence of 60 g/L CaCO₃.

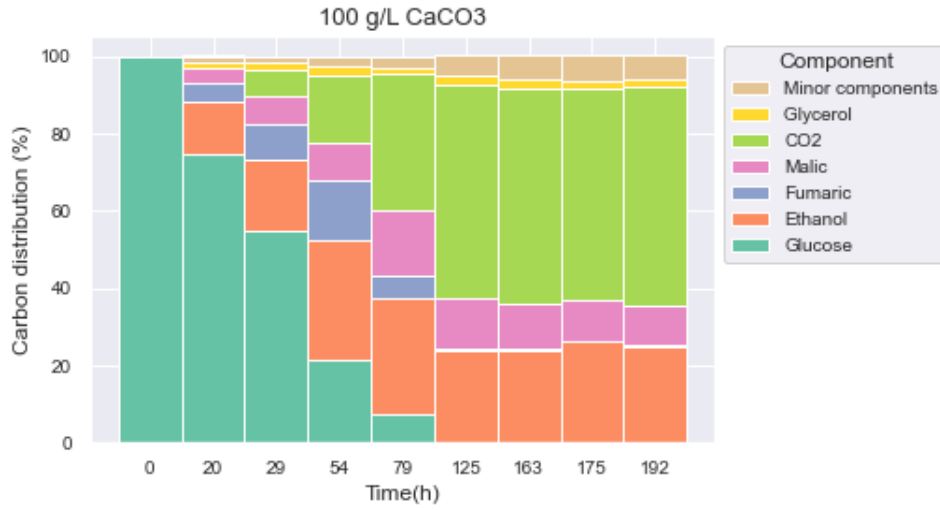


Figure S3. Metabolite distribution of *Rhizopus delemar* with the minor components including biomass, succinic acid and pyruvic acid. Cultures were incubated at 34 °C, 200 rpm in the presence of 60 g/L CaCO₃.

3 Glycerol profiles

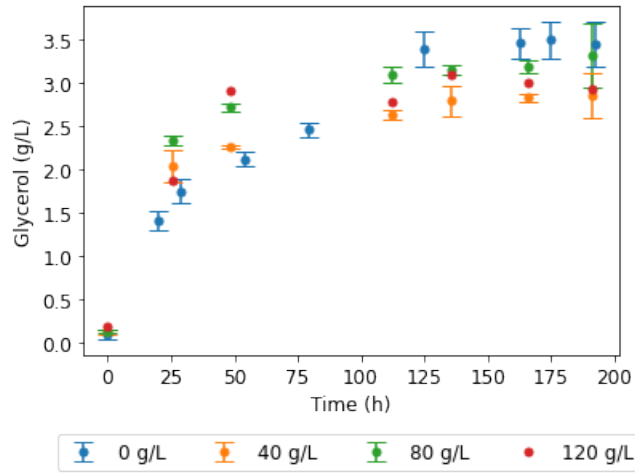


Figure S4. Extracellular concentrations of glycerol during shake flask cultivation of *R. delemar* with different plaster sand concentrations. Results are the mean of triplicate experiments and error bars indicate the standard deviation.

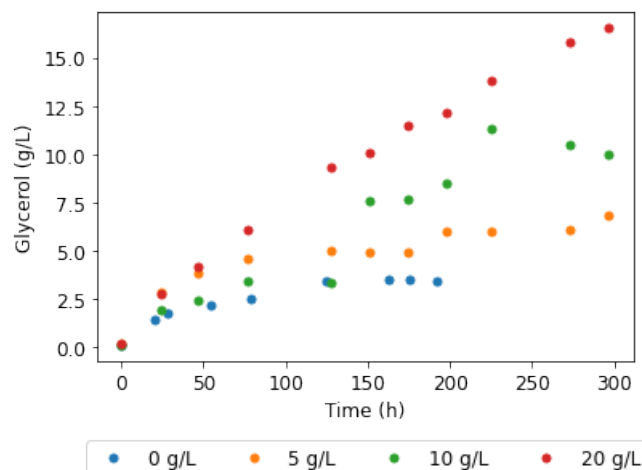


Figure S5. Extracellular concentrations of glycerol during shake flask cultivation of *R. delemar* with different calcium ion concentrations. 20 g L⁻¹ CaCO₃ was used for pH control as a baseline amount in these experiments and additional 5, 10 and 20 g L⁻¹ calcium ions were added onto the baseline in the form of CaCl₂.

References

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