

## Supplementary Material: Parameters sensitivity analysis.

To evaluate the influence and the effect of important parameters onto the model, different scenarios have been proposed, simulating the kinetic model through changes on the value of certain parameters. The simulations have been performed using experiment 4 as reference (the experiment using apple hydrolysate). The proposed scenarios are:

Scenario 1: Higher specific growth rate:  $\mu_m = 1.52 \text{ L}/(\text{g}_{\text{NH}_3} \cdot \text{h})$

In this scenario has been evaluated the influence of the specific growth rate. The value has been increased two times from that obtained in the kinetic modelling. In Figure 1 can be observed how increasing this value affects the production rate of ethanol, biomass, and the intermediate metabolite. At the same time, sugars and ammonia consumption rate are slightly increased too. On the other hand, fumaric and malic acids production rate are not apparently affected, such as their final concentration reached, not being affected their production yields.

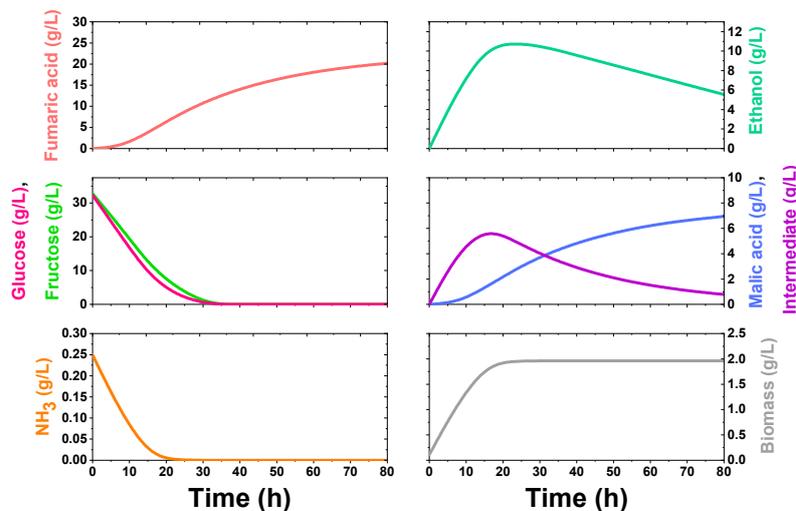


Figure S1: Scenario 1 simulation.

Scenario 2: APH totally detoxified:  $k_F = 0,29 \text{ L} \cdot \text{g}_{\text{Fumaric acid}}/(\text{g}_{\text{Biomass}} \cdot \text{g}_{\text{Sugar}} \cdot \text{h})$

This scenario evaluates the influence of  $k_F$  parameter on the final production yield. The chosen value of the parameter is the same as reached on synthetic medium with no inhibition on fumarase (whose behaviour is described by  $k_F$ ). In Figure 2 is appreciated how this parameter provokes a direct effect over the production yield, providing a higher final concentration of both, malic and fumaric acid, whose productions rates are

dependent of this parameter. Also, the evolution of the intermediate metabolite is affected too, having a faster consumption rate and a lower accumulation, reaching its maximum concentration in a shorter time with a lower value. This scenario has not effect on the evolution of the other components.

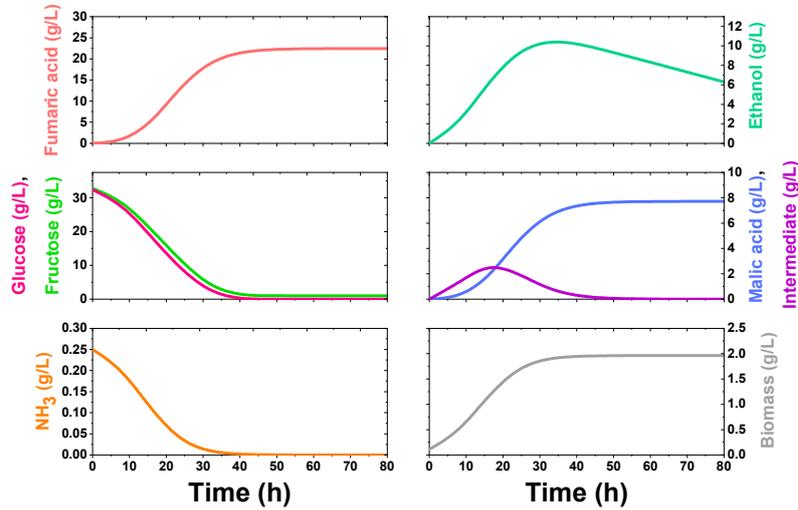


Figure S2: Scenario 2 simulation.

Scenario 3: Fermentation with no Crabtree effect:  $k_E = 0 \text{ g}_{\text{Ethanol}}/(\text{g}_{\text{Biomass}} \cdot \text{h})$ ;  $Y_{EX} = 0 \text{ g}_{\text{Ethanol}}/\text{g}_{\text{Biomass}}$

In bibliography has been demonstrated the possibility of removing the Crabtree effect. For this reason, this scenario has been simulated with the purpose to evaluate the influence of the production of ethanol and how its removal could affect to carbon flux in a way to improve the fumaric acid production. This scenario has been simulated reducing to zero the value of the parameters related with ethanol production ( $k_E$  and  $Y_{EX}$ ), at the same time sugars consumption yields have been reduced, due to the fact that ethanol production is totally associated to growth.

As can be seen in Figure 3, the only influence appreciated in the simulation is over sugars consumption, being this reduced. Despite of this, the sugar availability has not an appreciable effect on any production yield, neither biomass, intermediate metabolite nor fumaric or malic acids.

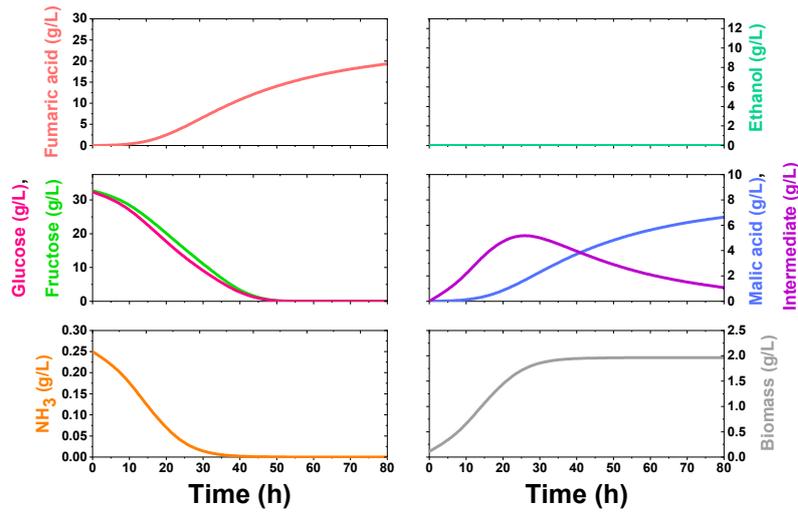


Figure S3: Scenario 3 simulation.

Scenario 4: Concentrated and totally detoxified APH:  $k_F = 0.29 \text{ L} \cdot \text{g}_{\text{Fumaric acid}} / (\text{g}_{\text{Biomass}} \cdot \text{g}_{\text{Sugar}} \cdot \text{h})$ ;  $[\text{Glucose}]_0 = 65 \text{ g/L}$ ;  $[\text{Fructose}]_0 = 65 \text{ g/L}$ ;  $[\text{NH}_3]_0 = 0.50 \text{ g/L}$

Finally, to simulate an improved APH concentrated and totally detoxified, initial values of glucose, fructose and ammonia concentrations have been modified in Scenario 2, obtaining the simulation represented in Figure 4. In this Figure, it can be observed how higher nutrients concentration increase consumption and production rates in the model. At the same time, final products concentration could increase, but the production yield is very similar to those obtained in Scenario 2 ( $0.34 \text{ g}_{\text{Fumaric acid}}/\text{g}_{\text{Consumed sugar}}$ ).

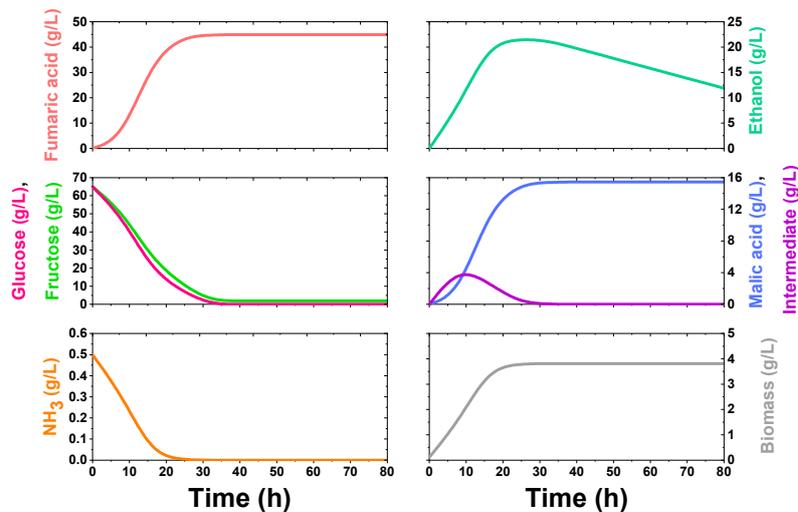


Figure S4: Scenario 4 simulation.