Supplementary Material

Sensitivity analysis

A sensitivity analysis (Equation S1) was applied for assessing the effect of the amino acid in-flux on the maximization of the objective function ($c^T v$), namely CA secretion and biomass maximization.

$$\begin{bmatrix} max/min\ c^T v \\ s.t. Sv = dxdt : y \\ c_v \leq d : y \\ lb \leq v \leq ub : w \end{bmatrix} \quad \text{(S1)}$$

The sensitivity analysis was performed in COBRA Toolbox v3.0 by using the robustness analysis function as follows, dividing the range of the uptake values [0,-0.1] in n = 20 intervals:

[controlFlux, objFlux] = robustnessAnalysis(model, 'EX_AA_e', nPoints)

where *model* is the previously loaded iDG1237 model, *'EX_AA_e'* is the id of the exchange reaction for the amino acid under study and *nPoints* is the number of intervals. Figs. S1A to S1E show the effect of varying the uptake flux of individual amino acids on the specific growth rate (h^{-1}) and CA secretion mmol/(g DCW.hr). The robustness analysis shows that arginine (Fig. 1D) and ornithine (Fig. 1B) could increase the carbon fluxes toward biomass and CA biosynthesis in a slightly higher proportion than threonine (Fig. 1C), proline (Fig. 1E) and glutamic acid (Fig. 1A).

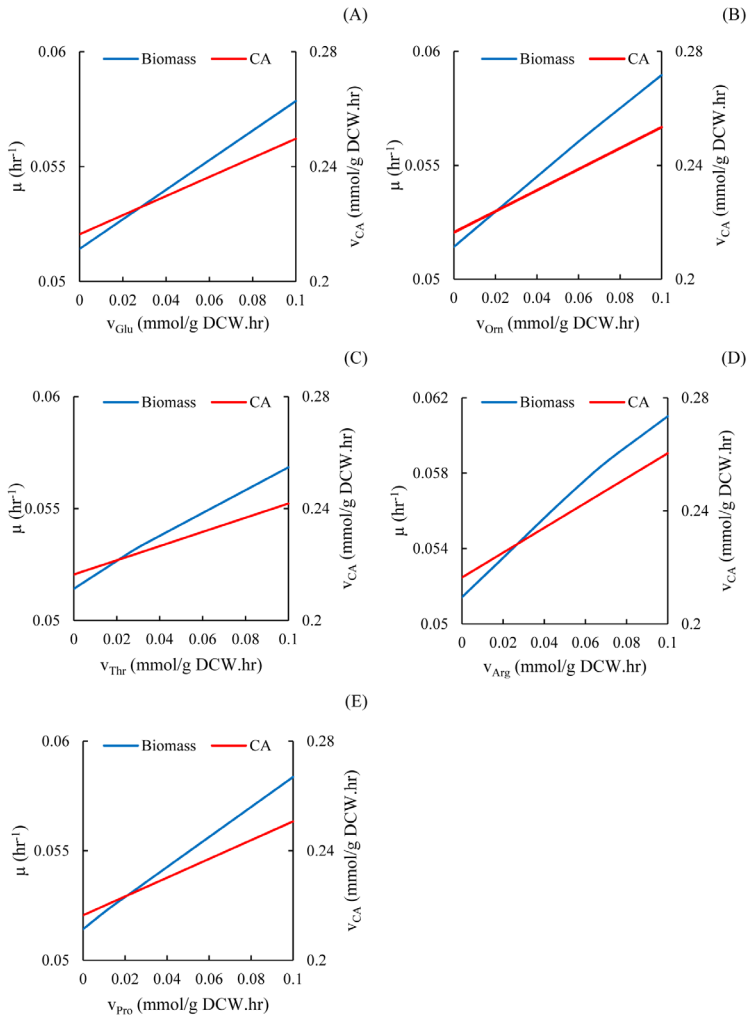

Fig. S1 Robustness analysis of amino acids uptake in growth (μ) and clavulanic acid (CA) secretion objective functions: (A) glutamate ($_{Glu}$); (B) ornithine ($_{Orn}$); (C) threonine ($_{Thr}$); (D) arginine ($_{Arg}$); (E) proline ($_{Pro}$), where DCW = dry cell weight, V_{CA} = clavulanic acid secretion flux and V_{Glu} = glutamate uptake flux for glutamate and the other specified amino acids

Effect of agitation on S. clavuligerus cultures

Supplementary Figures

The k_La can be enhanced by increasing the oxygen dissolution into the liquid phase by larger agitation rates[1]. Nevertheless, excessive shear stress could negatively affect *S. clavuligerus* growth. The effect of agitation on biomass and CA production was explored by performing fed-batch cultivations of *S. clavuligerus* using the RMO medium and modifying the agitation rates from 220 to 250 rpm. The time courses of biomass and CA at 200, 220 and 250 rpm are displayed in Fig S2.

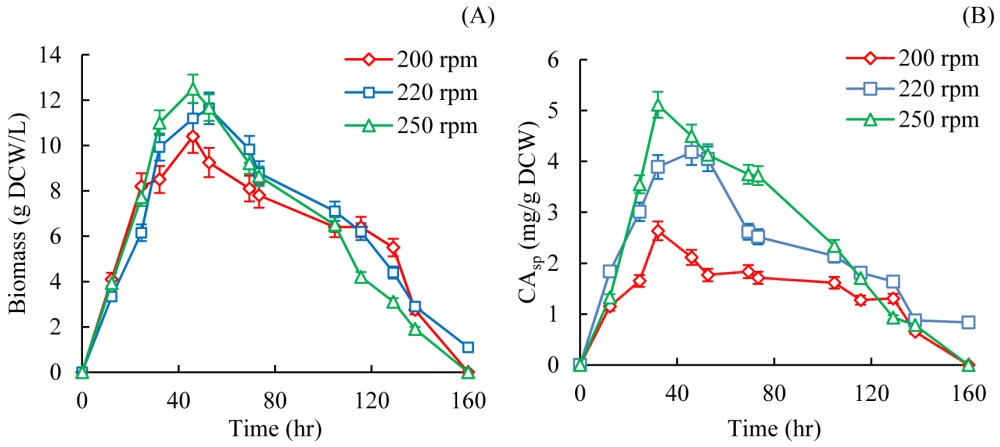

Fig. S2 Effect of agitation rates in *S. clavuligerus* fed-batch cultures at 200 revolutions per minute (rpm), 220 rpm and 250 rpm: (A) biomass concentration; (B) specific clavulanic acid concentration (CA_{sp}), where DCW = dry cell weight and error bars indicate ± SD

[1] Rosa, J.C., Neto, A.B., Hokka, C.O., Badino, A.C. 2005. Influence of dissolved oxygen and shear conditions on clavulanic acid production by *Streptomyces clavuligerus*. Bioprocess Biosyst. Eng. 27: 99–104. doi.org/10.1007/s00449-004-0386-9