Supporting Information

For

Internal mass transfer considerations in biofilms of succinic acid producing *Actinobacillus succinogenes*

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Table of Contents

S – 1 Discretisation of the pseudo-steady state reactor model

Under pseudo-steady state conditions, there is no mass accumulation within the biofilm layer, and as such the rate of reaction equals the rate of diffusion at any point in the biofilm. Equation S1 gives the general mass balance used to solve for the concentration profiles.

$$D_{e_j} \frac{d^2 C_j}{dz^2} = r_j' C_X \tag{S1}$$

The second order differential equations in the pseudo-steady state model (equations were discretised using a central finite difference method. As an example, the discretisation of the SA mass balance leads to,

$$z_0 = 0, \dots, z_i = i\Delta z, \dots, z_n = L = n\Delta z$$

$$\frac{d^2 C_{SA}}{dz^2} \approx \frac{C_{SA_{i+1}} - 2C_{SA_i} + C_{SA_{i-1}}}{\Delta z^2} \tag{S2}$$

By applying these transformations to equation 10 for i = 1 to i = n -1, we get the discretised system presented in equation S2 and S3

$$De_{SA} \frac{C_{SA_{i+1}} - 2C_{SA_i} + C_{SA_{i-1}}}{\Delta z^2} = \left(\alpha 0.82 \left(1 - e^{-6e^{-0.54 C_{SA}i}}\right) + \frac{k C_{SA_i}}{K_P + C_{SA_i} + \frac{C_{SA_i}^2}{K_I}} \right) C_X \tag{S3}$$

$$C_{SA_{i+1}} = 2C_{SA_i} - C_{SA_{i-1}} + \left(\alpha 0.82 \left(1 - e^{-6e^{-0.54 C_{SA}i}}\right) + \frac{k C_{SA_i}}{K_P + C_{SA_i} + \frac{C_{SA_i}^2}{K_I}} \right) C_X \frac{\Delta z^2}{De_{SA}} \tag{S4}$$

With the following boundary conditions,

$$\begin{cases} C_{SA_n} = C_{SA_{aq}} & succinic\ acid\ concentration\ in\ the\ bulk\ conditions \\ C_{SA_1} = C_{SA_0} & so\ that\ the\ flux\ at\ the\ solid - biofilm\ interface\ is\ zero \end{cases}$$

A second order Newton-Raphson method is consequently employed to solve the n -1 system of equations with n -1 unknown variables.

S – 2 Discretisation of the batch reactor model

The batch system consists of the bulk liquid phase and the biofilm phase. In the liquid phase suspended cells grow and convert the substrate to products and there is diffusion of metabolic products and the substrate out of and into the biofilm phase, respectively. Equation 1 and 2 give a generic mass balance in the liquid phase.

$$\frac{dC_{jaq}}{dt} = r_j' C_{X,aq} + J_j \tag{S5}$$

Where the mass flux Jj, is given by Equation 2 which can

$$J_j = D_{e-j} A_p \frac{dC_j}{dz}|_{z=L} \tag{S6}$$

In the biofilm phase, the general mass balance is presented in Equation 15

$$\frac{dC_j}{dt}|_z = -D_{e-j} \frac{d^2 C_j}{dz^2} + r_j' C_{XB} \tag{S7}$$

First order and second order differential equations were discretised using the central difference method in order to numerically solve the batch system of equations numerically with an iterative process that uses a finite difference method. Time and the biofilm thickness domains were first discretised as follows:

$$z_0 = 0, \ldots, z_i = i\Delta z, \ldots, z_n = L = n\Delta z$$

$$t_0 = 0, \ldots, t_m = m\Delta t, \ldots, t_p = T = p\Delta t$$

Using SA as an example, Equation (S7) for the general mass balance in the biofilm layer is discretised as follows:

$$\frac{C_{SA_i}^{m+1}-C_{SA_i}^{m}}{\Delta t} = -De_{SA}\frac{C_{SA_{i+1}}^{m}-2C_{SA_i}^{m}+C_{SA_{i-1}}^{m}}{\Delta z^2} + \left(\alpha\mu + \frac{kC_{SA_i}^{m}}{K_P + C_{SA_i}^{m} + \frac{\left(C_{SA_i}^{m}\right)^2}{K_I}}\right)C_{XB} \quad \text{(S8)}$$

Rearranging equation S8, we end up with a discretised equation S9 which solves for the mass balance in the biofilm phase of the system.

$$C_{SA_i}^{m+1} = C_{SA_i}^{m} + \left(C_{SA_{i+1}}^{m} - 2C_{SA_i}^{m} + C_{SA_{i-1}}^{m}\right)\left(\frac{-De_{SA}\Delta t}{\Delta z^2}\right) + \left(\alpha\mu + \frac{kC_{SA_i}^{m}}{K_P + C_{SA_i}^{m} + \frac{\left(C_{SA_i}^{m}\right)^2}{K_I}}\right)\Delta t C_{XB} \quad \text{(S9)}$$

Then equation (S5) and (S6) are combined and discretised as follows to give the general mass balance for the bulk liquid phase

$$\frac{C_{SA,aq}^{m+1}-C_{SA,aq}^{m}}{\Delta t} = \left(\alpha\mu + \frac{kC_{SA,aq}^{m}}{K_P + C_{SA,aq}^{m} + \frac{\left(C_{SA,aq}^{m}\right)^2}{K_I}}\right)C_{X,aq} + D_{e_{SA}}A_p\left(\frac{C_{SA_n}-C_{SA_{n-1}}}{\Delta z}\right) \quad \text{(S10)}$$

Finally, the general mass balance in the bulk liquid phase becomes.

$$C_{SA,aq}^{m+1} = C_{SA,aq}^{m} + \left(\alpha\mu + \frac{kC_{SA,aq}^{m}}{K_P + C_{SA,aq}^{m} + \frac{\left(C_{SA,aq}^{m}\right)^2}{K_I}}\right)\Delta t C_{X,aq} + D_{e_{SA}}A_p\Delta t\left(\frac{C_{SA_n}-C_{SA_{n-1}}}{\Delta z}\right) \quad \text{(S11)}$$

S – 3 Continuous pseudo steady state data obtained from literature

Table S – 1 : Continuous fermentation pseudo-steady state data used to analyse internal mass in the tubular biofilm reactor, source: Maharaj et al. [9], Brink & Nicol [10,26], and Mokwatlo et al. [21].

Dilution rate (h^{-1})	$C_{Glc,\ aq}$ ($g.L^{-1}$)	ΔGlc ($g.L^{-1}$)	$C_{SA,aq}$ ($g.L^{-1}$)	$C_{AA,aq}$ ($g.L^{-1}$)	$C_{FA,aq}$ ($g.L^{-1}$)	Total dry Biomass ($g.L^{-1}$)	Total dry cell biomass ($g.L^{-1}$)	Total dry EPS ($g.L^{-1}$)	L (μm)	Shear velocity ($m.s^{-1}$)	Source
0.054	15.3	36.1	32.5	5.6	2.3	23.8	11.9	11.9	575.4	0.04	[9]
0.11	17.8	37.4	31.4	5.1	2.4	27.9	14.0	14.0	674.5		
0.11	18.5	31.5	28.0	5.3	2.5	23.8	11.9	11.9	575.4		
0.11	19.1	32.8	29.5	3.4	1.3	23.8	11.9	11.9	575.4		
0.31	11.2	23.8	20.2	4.6	3.0	23.8	11.9	11.9	575.4		
0.31	9.8	25.1	21.6	6.3	3.8	23.8	11.9	11.9	575.4		
0.31	32.7	23.4	19.4	5.5	3.3	23.8	11.9	11.9	575.4		
0.32	6.9	26.7	22.2	3.2	1.6	23.8	11.9	11.9	575.4		
0.32	8.1	25.7	20.8	4.2	2.9	23.8	11.9	11.9	575.4		
0.52	17.1	17.3	13.7	4.6	2.1	23.8	11.9	11.9	575.4		
0.71	17.0	17.6	14.1	4.2	3.2	23.8	11.9	11.9	575.4		
0.72	19.1	15.6	12.0	4.0	2.0	23.8	11.9	11.9	575.4		
0.72	18.3	13.8	11.3	4.4	3.5	23.8	11.9	11.9	575.4		
0.51	14.9	26.1	18.2	5.56	2.26	26.1	13.1	13	309	0.09	[10]

0.55	19	21.9	15.6	5.12	2.44	28.9	18.9	10	347		
0.61	18.8	22.8	15.9	5.29	2.47	28.3	16.7	11.6	338		
0.71	26.5	15.1	11.3	3.4	1.29	15.5	8.8	6.7	175		
0.93	28.3	13.1	9.72	3.23	1.64	15	8.2	6.8	169		
0.98	23.1	15.2	10.4	4.21	2.86	17	9.9	7.1	193		
1.0	21	17.1	12.3	4.59	3.01	27.3	17.5	9.8	325		
0.44	13.6	26.7	17.6	6.31	3.79	14.2	11	3.2	159	0.36	[26]
0.75	20.7	19.6	13.7	5.48	3.32	22	16.9	5.1	255		
0.77	22.1	18.6	12.8	4.56	2.13	15.2	12.6	2.6	171		
1.0	25.4	16.4	9.5	4.18	3.19	18.9	15.4	3.5	216		
1.2	24.1	17.1	11.5	4.01	2.02	17.8	16.5	1.3	203		
1.2	25.3	15.5	9.65	4.36	3.46	16.6	11.7	4.9	188		
1.4	23.3	18	10.9	4.31	2.93	14.2	13.5	0.7	159		
0.9	19.9	20.1	11.6	4.77	2.4	15.4	13.1	2.39	258	0.36	[21]
0.9	15.8	24.2	14.9	5.6	2.2	11.7	5.38	6.31	196	0.64	

S – 4 Theoretically generated data

Table S - 2: Realistic pseudo-steady state data generated for a hypothetical *A. succinogenes* biofilm

$C_{SA,aq}$ ($g.L^{-1}$)	$C_{Glc,aq}$ ($g.L^{-1}$)	C_{XB} ($g.L^{-1}$)	L^* (μm)	ε_{cells}	ε_{EPS}
10	86.6	57.6	834	0.5	0.3
25	68.2	57.6	790	0.5	0.3
45	47.0	57.6	740	0.5	0.3
10	86.6	34.2	730	0.3	0.5
25	68.2	34.2	695	0.3	0.5
25	68.2	57.6	200	0.5	0.3
25	68.2	57.6	400	0.5	0.3
25	68.2	57.6	600	0.5	0.3
25	68.2	57.6	790	0.5	0.3
25	68.2	57.6	790	0.5	0.3
45	47.0	57.6	200	0.5	0.3
45	47.0	57.6	400	0.5	0.3
45	47.0	57.6	600	0.5	0.3
25	68.2	10.9	500	0.1	0.7
25	68.2	22.6	500	0.2	0.6
25	68.2	34.2	500	0.3	0.5
25	68.2	45.9	500	0.4	0.4
25	68.2	57.6	500	0.5	0.3
45	47.0	10.9	500	0.1	0.7
45	47.0	22.6	500	0.2	0.6
45	47.0	34.2	500	0.3	0.5
45	47.0	45.9	500	0.4	0.4
45	47.0	57.6	500	0.5	0.3
25	68.2	61.5	500	0.53	0.267
25	68.2	61.5	500	0.57	0.283
25	68.2	61.5	500	0.6	0.3

25	68.2	61.5	500	0.63	0.317
25	68.2	61.5	500	0.65	0.325
25	68.2	22.6	500	0.2	0.6
25	68.2	22.6	500	0.213	0.6375
25	68.2	22.6	495	0.225	0.675
25	68.2	22.6	352	0.238	0.7125
25	68.2	22.6	250	0.244	0.73125
25	68.2	8.5	500	0.08	0.72
25	68.2	8.5	500	0.085	0.765
25	68.2	8.5	500	0.09	0.81
25	68.2	8.5	500	0.095	0.855

S – 5 Interaction between system variables (L^*, $D_{e\text{-}SA}$, C_{SA}, C_{XB}) and biofilm effectiveness (η)

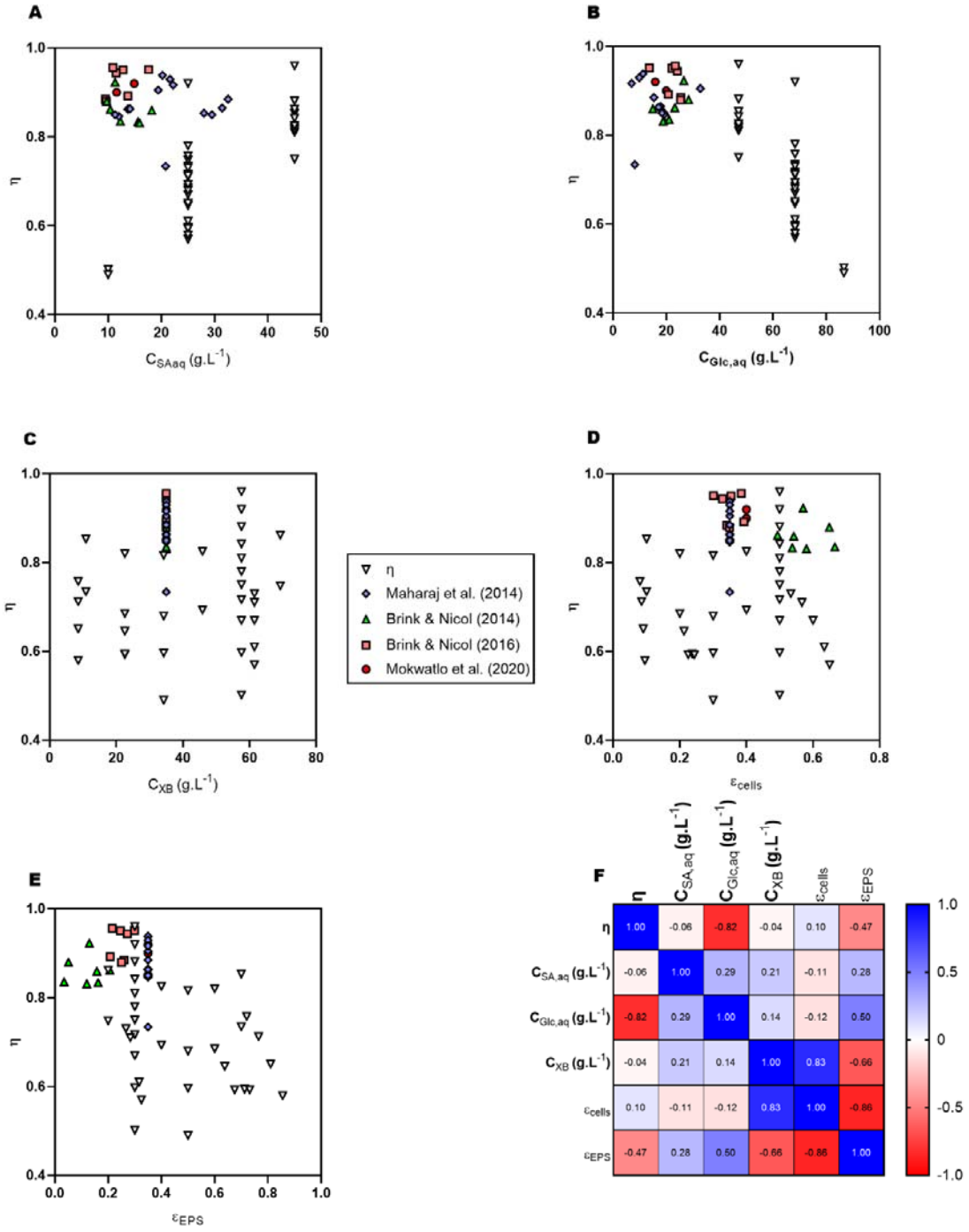

Figure S - 1: The relationships between the system parameters (A: $C_{SA,aq}$, B: $C_{Glc,aq}$. C: C_{XB}, D: ε_{cells}, E: ε_{EPS}) and η (determined from the IMT model). F shows the Pearson correlations between the respective parameters.