applied sciences

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Supplementary Material 3

Modelling of the acetification stage in the production of wine vinegar by use of two serial bioreactors

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This Supplementary Material describes the procedure used to obtain each of the polynomial models presented in the Results and Discussion section.

The results were initially subjected to ANOVA in order to identify statistically significant differences (i.e., differences not exclusively due to experimental error) between experiments. Then, the Forward Stepwise Regression method, described in the Material and Methods section, as implemented in the software SigmaStat 2.0 (Systat Software Inc., 2015) was used for fitting. A total of 27 terms (viz., 6 for the operational variables and 21 for their mutual interactions) were initially used even though the greatest number of terms in each polynomial could not exceed the number of experiments: 18.

Significant differences between the regression for each step of the process and the previous one by effect of the addition of new terms or removal of existing ones were sought by ANOVA. Because the differences were all significant at the 95% probability level, the results of the ANOVA are not shown here.

S3.1. Mean overall rate of acetic acid formation in the two-bioreactor system

The experimental results of the ANOVA on this variable are shown in Table S3.1.

Table S3.1. Results of the ANOVA on the mean rate of acetic acid formation in the two-bioreactor system.

Source of variability	Degrees of freedom	Variance	*F*	*P-value*
Group	17	0.0098	97.977	<0.001
Sample	144	0.0001		
Total	161			

The F value at the 95% confidence level was greater than the corresponding critical point (F_{crit} = 1.623), so there were statistically significant differences between experimental means (*P-value* < 0.001).

The Forward Stepwise Regression method was applied stepwise, the polynomials being expanded with those terms having the smallest *P*-value and greatest F value exceeding *F*-to-enter or contracted by removing those with an F value smaller than *F*-to-remove (see Section 2). *F*-to-enter was set at 4 (*P-value* = 0.051) and *F*-to-remove at 3.9 (*P-value* = 0.054). Each step was followed by an ANOVA intended to expose significant differences between the predictions of the model for that step and the previous one. If the null hypothesis for the test could not be rejected or no term with $F > F$-to-enter was found, then the fitting was finished. Tables S3.2 to S3.14 show the results of each step.

Note that Step 0 invariably started by incorporating the constant term of the polynomial (see eq. 1). The terms added in each step are shown in successive rows in each table.

Table S3.2. Results of Step 0 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	Coefficient	Standard error
Constant	0.171	
		0.0317

Table S3.3. Results of Step 1 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		0.125			
T_1E_{u1}	14.71	0.00051			
			0.0283	0.470	0.221

Table S3.4. Results of Step 2 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		0.128			
T_1E_{u1}	29.685	0.00138			
$E_{u1}V_{u1}$	15.039	-0.00543			
			0.0251	0.631	0.398

Table S3.5. Results of Step 3 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		0.0736			
T_1E_{u1}	23.419	0.00255			
$E_{u1}V_{u1}$	7.812	-0.00404			
E_{u1}^2	6.271	-0.0073			
			0.0239	0.682	0.465

Table S3.6. Results of Step 4 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-0.401			
T_1E_{u1}	32.001	0.00174			
$E_{u1}V_{u1}$	38.863	-0.00499			
E_{u1}^2	124.98	-0.0654			
E_{u1}	107.162	0.379			
			0.0135	0.912	0.832

Table S3.7. Results of Step 5 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-0.436			
T_1E_{u1}	24.909	0.00139			
$E_{u1}V_{u1}$	50.717	-0.00506			
E_{u1}^2	167.788	-0.0654			
E_{u1}	151.242	0.39			

T_1E_{l1}	17.783	0.000235			
			0.0117	0.937	0.878

Table S3.8. Results of Step 6 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-0.534			
T_1E_{u1}	1.996	0.000414			
$E_{u1}V_{u1}$	83.465	-0.00523			
E_{u1}^2	258.949	-0.0654			
E_{u1}	256.947	0.453			
T_1E_{l1}	44.409	0.000887			
$E_{u1}E_{l1}$	27.079	-0.00662			
			0.00939	0.960	0.922

As can be seen from Table S3.8, the *F* value for the term T_1E_{u1} was less than the preset *F*-to-remove value (3.9), so the term was removed from the polynomial in the following step.

Table S3.9. Results of Step 7 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-0.553			
$E_{u1}V_{u1}$	82.989	-0.00526			
E_{u1}^2	253.683	-0.0654			
E_{u1}	345.574	0.471			
T_1E_{l1}	106.506	0.00102			
$E_{u1}E_{l1}$	62.234	-0.00777			
			0.00948	0.959	0.919

Table S3.10. Results of Step 8 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-0.528			
$E_{u1}V_{u1}$	5.862	-0.00279			
E_{u1}^2	279.705	-0.0654			
E_{u1}	313.563	0.451			
T_1E_{l1}	117.084	0.00112			
$E_{u1}E_{l1}$	30.981	-0.00626			
$E_{l1}V_{u1}$	5.924	-0.00167			
			0.00903	0.963	0.928

Table S3.11. Results of Step 9 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-0.534			
$E_{u1}V_{u1}$	7.075	-0.00296			
E_{u1}^2	302.772	-0.0654			
E_{u1}	339.204	0.451			
T_1E_{l1}	113.912	0.00108			
$E_{u1}E_{l1}$	31.315	-0.00607			
$E_{l1}V_{u1}$	5.517	-0.00155			

$E_{l2}T_1$	4.876	0.0000911			
			0.00868	0.967	0.935

Table S3.12. Results of Step 10 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-0.525			
$E_{u1}V_{u1}$	2.779	-0.00185			
E_{u1}^2	348.659	-0.0654			
E_{u1}	370.659	0.443			
T_1E_{l1}	20.952	0.000726			
$E_{u1}E_{l1}$	29.138	-0.00555			
$E_{l1}V_{u1}$	0.0826	0.000255			
$E_{l2}T_1$	10.297	0.000675			
$E_{l2}V_{u1}$	7.972	-0.00354			
			0.00868	0.967	0.935

As can be seen from Table S3.12, two terms had an *F* value smaller than *F*-to-remove. Therefore, the term with the smaller *F* value (viz., $E_{l1}V_{u1}$) was removed in the following step.

Table S3.13. Results of Step 11 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-0.524			
$E_{u1}V_{u1}$	2.815	-0.00175			
E_{u1}^2	355.754	-0.0654			
E_{u1}	383.142	0.442			
T_1E_{l1}	55.538	0.00076			
$E_{u1}E_{l1}$	30.742	-0.00548			
$E_{l2}T_1$	19.019	0.000632			
$E_{l2}V_{u1}$	14.532	-0.00328			
			0.00801	0.972	0.945

As can be seen, Table S3.13 contained a term with *F* < *F*-to-remove (viz., $E_{u1}V_{u1}$, which was thus discarded in Step 12).

Table S3.14. Results of Step 12 in the fitting of the mean rate of acetic acid formation in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-0.51			
E_{u1}^2	342.526	-0.0654			
E_{u1}	392.086	0.43			
T_1E_{l1}	56.948	0.000672			
$E_{u1}E_{l1}$	28.095	-0.00468			
$E_{l2}T_1$	119.001	0.000839			
$E_{l2}V_{u1}$	122.922	-0.00456			
			0.00816	0.970	0.941

Beyond this point, no term had *F* > *F*-to-enter so the polynomial was not further expanded and the fitting process was finished. The final polynomial is that of eq. 5 of the manuscript.

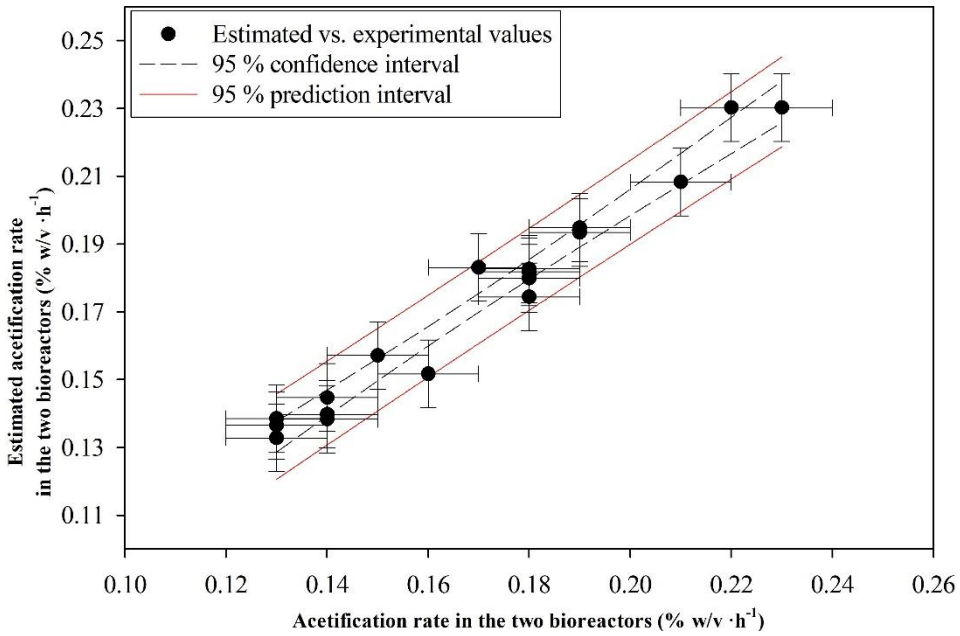

Figure S3.1. Plot of $(r_A)_{global\ est}$ against $(r_A)_{global}$ and curves for the 95% confidence and prediction intervals.

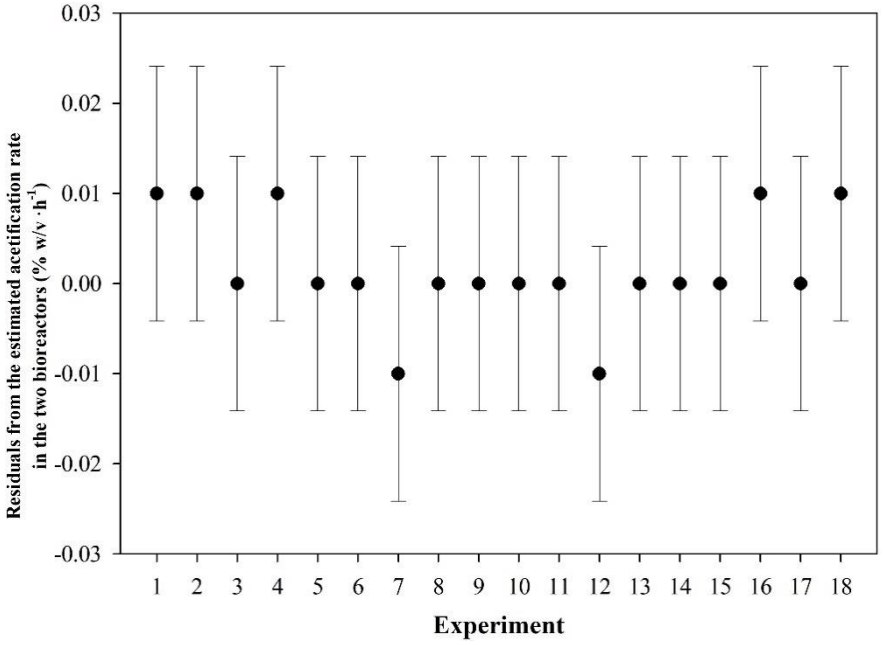

Figure S3.2. Residuals of the fitting of $(r_A)_{global\ est}$ for each experiment.

S3.2. Total acetic acid production in the two-bioreactor system

The experimental results of the ANOVA on this variable are shown in Table S3.15.

Table S3.15. Results of the ANOVA on the total acetic acid production in the two-bioreactor system.

Source of variability	Degrees of freedom	Variance	*F*	*P-value*
Group	17	193.059	326.795	<0.001
Sample	144	0.591		
Total	161			

The F value at the 95% confidence level was greater than the corresponding critical point (F_{crit} = 1.623), so there were statistically significant differences between experimental means (*P-value* < 0.001).

The Forward Stepwise Regression method was applied. F-to-enter was set at 4 and F-to-remove at 3.9. Tables S3.16 to S3.28 show the results of each step. The terms added in each step are shown in successive rows in each table.

Table S3.16. Results of Step 0 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	Coefficient	Standard error
Constant	22.445	
		4.231

Table S3.17. Results of Step 1 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		12.485			
T_1E_{l1}	15.002	0.0664			
			3.763	0.473	0.224

Table S3.18. Results of Step 2 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		5.45			
T_1E_{l1}	12.132	0.177			
E_{l1}^2	5.306	-0.372			
			3.616	0.545	0.297

Table S3.19. Results of Step 3 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-220.174			
T_1E_{l1}	27.548	0.148			
E_{l1}^2	127.466	-9.708			
E_{l1}	119.158	94.363			
			1.986	0.890	0.792

Table S3.20. Results of Step 4 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-223.529			
T_1E_{l1}	34.781	0.146			
E_{l1}^2	163.678	-9.708			
E_{l1}	153.13	94.4			
T_2E_{l2}	15.205	0.032			
			1.986	0.917	0.841

Table S3.21. Results of Step 5 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-217.918			
T_1E_{l1}	57.572	0.182			
E_{l1}^2	207.527	-9.708			
E_{l1}	189.413	93.324			
T_2E_{l2}	19.096	0.0318			
T_1V_{u1}	14.127	-0.0373			
			1.556	0.937	0.877

Table S3.22. Results of Step 6 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-218.368			
T_1E_{l1}	40.423	0.496			
E_{l1}^2	278.459	-9.708			
E_{l1}	178.929	83.914			
T_2E_{l2}	23.486	0.0305			
T_1V_{u1}	21.359	-0.363			
V_{u1}	17.406	9.902			
			1.344	0.954	0.911

Table S3.23. Results of Step 7 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-219.721			
T_1E_{l1}	44.712	0.496			
E_{l1}^2	308.001	-9.708			
E_{l1}	197.911	83.914			
T_2E_{l2}	25.978	0.0305			
T_1V_{u1}	23.625	-0.363			
V_{u1}	19.253	9.902			
E_{u1}	5.986	0.451			
			1.277	0.960	0.921

Table S3.24. Results of Step 8 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-220.023			
T_1E_{l1}	48.403	0.494			
E_{l1}^2	335.979	-9.708			
E_{l1}	216.172	83.97			
T_2E_{l2}	32.359	0.0334			
T_1V_{u1}	25.511	-0.362			
V_{u1}	20.769	9.847			
E_{u1}	7.531	2.403			
T_2E_{u1}	5.179	-0.065			
			1.223	0.964	0.929

Table S3.25. Results of Step 9 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-219.853			
T_1E_{l1}	84.488	0.495			
E_{l1}^2	583.992	-9.708			
E_{l1}	302.445	77.646			
T_2E_{l2}	50.719	0.0317			
T_1V_{u1}	44.594	-0.363			
V_{u1}	36.325	9.878			
E_{u1}	46.083	11.79			
T_2E_{u1}	42.879	-0.378			
T_2E_{l1}	34.218	0.21			
			0.928	0.980	0.960

Table S3.26. Results of Step 10 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-225.816			
T_1E_{l1}	79.04	0.458			
E_{l1}^2	679.143	-9.708			
E_{l1}	355.655	80.793			
T_2E_{l2}	19.028	0.0885			
T_1V_{u1}	39.661	-0.327			
V_{u1}	31.615	8.803			
E_{u1}	57.628	12.301			
T_2E_{u1}	53.795	-0.395			
T_2E_{l1}	27.31	0.181			
$E_{l2}E_{l1}$	8.169	-0.342			
			0.860	0.983	0.966

Table S3.27. Results of Step 11 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-240.827			
T_1E_{l1}	82.379	0.67			
E_{l1}^2	872.78	-9.708			
E_{l1}	331.778	74.894			
T_2E_{l2}	30.908	0.101			
T_1V_{u1}	53.475	-0.534			
V_{u1}	39.664	17.749			
E_{u1}	78.115	16.969			
T_2E_{u1}	70.463	-0.399			
T_2E_{l1}	32.527	0.175			
$E_{l2}E_{l1}$	15.018	-0.416			
$E_{u1}V_{u1}$	13.26	-0.91			
			0.759	0.987	0.975

Table S3.28. Results of Step 12 in the fitting of the total acetic acid production in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		-243.705			
T_1E_{l1}	92.918	0.742			
E_{l1}^2	961.793	-9.708			
E_{l1}	326.099	72.736			
T_2E_{l2}	34.06	0.101			
T_1V_{u1}	58.929	-0.534			
V_{u1}	46.188	18.324			
E_{u1}	63.689	21.525			
T_2E_{u1}	77.65	-0.399			
T_2E_{l1}	35.845	0.175			
$E_{l2}E_{l1}$	16.55	-0.416			
$E_{u1}V_{u1}$	19.078	-1.102			
T_1E_{u1}	5.284	-0.12			
			0.723	0.989	0.977

Beyond this point, no term had *F* > *F*-to-enter so the polynomial was not further expanded and the fitting process was finished. The final polynomial is that of eq. 2 of the manuscript.

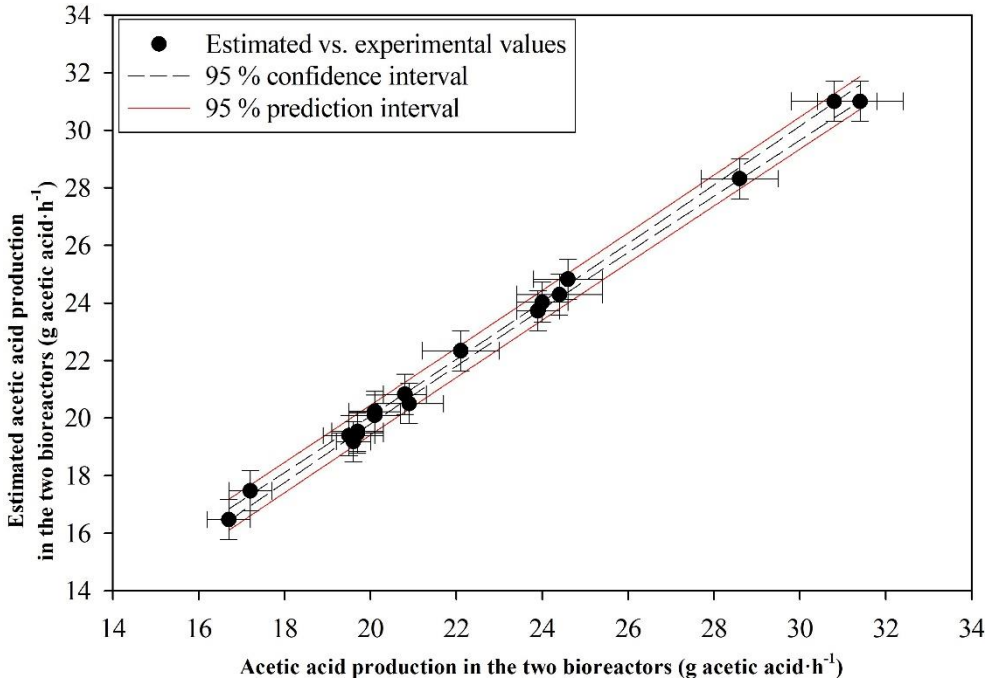

Figure S3.3. Plot of $P_{m\,est}$ against P_m and curves for the 95% confidence and prediction intervals.

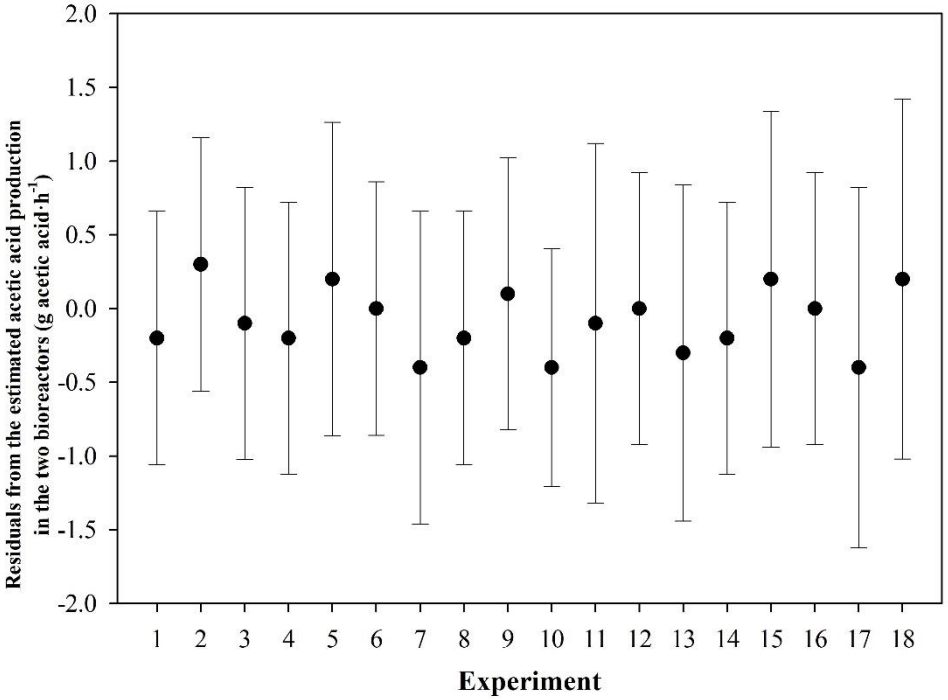

Figure S3.4. Residuals of the fitting of $P_{m\,est}$ for each experiment.

S3.3. Final ethanol concentration at the time the second reactor was unloaded

The experimental results of the ANOVA on this variable are shown in Table S3.29.

Table S3.29. Results of the ANOVA on the final ethanol concentration at the time the second reactor was unloaded.

Source of variability	Degrees of freedom	Variance	*F*	*P-value*
Group	17	21.36	545.372	<0.001
Sample	144	0.0392		
Total	161			

The *F* value at the 95% confidence level was greater than the corresponding critical point (F_{crit} = 1.623), so there were statistically significant differences between experimental means (*P-value* < 0.001).

The Forward Stepwise Regression method was applied. *F*-to-enter was set at 4 and *F*-to-remove at 3.9. Tables S3.30 to S3.41 show the results of each step. The terms added in each step are shown in successive rows in each table.

Table S3.30. Results of Step 0 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	Coefficient	Standard error
Constant	1.329	
		0.202

Table S3.31. Results of Step 1 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		8.834			
T_2V_{u1}	86.921	-0.05			
			0.917	0.791	0.626

Table S3.32. Results of Step 2 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		8.01			
T_2V_{u1}	102.461	-0.0502			
$E_{l2}E_{u1}$	9.819	0.0813			
			0.848	0.828	0.686

Table S3.33. Results of Step 3 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		7.658			
T_2V_{u1}	63.213	-0.0415			
$E_{l2}E_{u1}$	22.903	0.143			
$E_{l2}V_{u1}$	11.42	-0.092			
			0.773	0.863	0.744

Table S3.34. Results of Step 4 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		6.045			
T_2V_{u1}	3.733	-0.0122			
$E_{l2}E_{u1}$	67.914	0.391			
$E_{l2}V_{u1}$	54.156	-0.222			
$E_{u1}V_{u1}$	35.72	-0.207			
			0.594	0.923	0.852

T_2V_{u1} must be removed from the model in the next step.

Table S3.35. Results of Step 5 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		5.043			
$E_{l2}E_{l1}$	218.352	0.462			
$E_{l2}V_{u1}$	187.843	-0.268			
$E_{u1}V_{u1}$	131.888	-0.258			
			0.610	0.917	0.841

Table S3.36. Results of Step 6 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		7.08			
$E_{l2}E_{u1}$	282.269	0.637			
$E_{l2}V_{u1}$	25.252	-0.135			
$E_{u1}V_{u1}$	200.501	-0.382			
T_2E_{l2}	35.656	-0.0414			
			0.469	0.953	0.908

Table S3.37. Results of Step 7 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		7.048			
$E_{l2}E_{u1}$	81.703	0.462			
$E_{l2}V_{u1}$	0.197	-0.0157			
$E_{u1}V_{u1}$	159.136	-0.542			
T_2E_{l2}	53.649	-0.0434			
E_{u1}^2	19.464	0.242			
			0.399	0.967	0.935

$E_{l2}V_{u1}$ must be removed from the model in the next step.

Table S3.38. Results of Step 8 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		7.089			
$E_{l2}E_{u1}$	96.335	0.453			
$E_{u1}V_{u1}$	542.537	-0.558			
T_2E_{l2}	90.225	-0.045			
E_{u1}^2	54.936	0.261			
			0.396	0.967	0.934

Table S3.39. Results of Step 9 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		13.357			
$E_{l2}E_{u1}$	171.372	0.485			
$E_{u1}V_{u1}$	810.614	-0.545			
T_2E_{l2}	160.166	-0.0481			
E_{u1}^2	52.77	0.988			
E_{u1}	29.857	-4.557			
			0.314	0.980	0.959

Table S3.40. Results of Step 10 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		13.928			
$E_{l2}E_{u1}$	194.067	0.488			
$E_{u1}V_{u1}$	546.045	-0.513			
T_2E_{l2}	181.617	-0.0484			
E_{u1}^2	59.082	0.988			
E_{u1}	35.746	-4.729			
$E_{l1}V_{u1}$	6.741	-0.0214			
			0.297	0.982	0.965

Table S3.41. Results of Step 11 in the fitting of the final ethanol concentration at the time the second reactor was unloaded.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		14.935			
$E_{l2}E_{u1}$	211.593	0.494			
$E_{u1}V_{u1}$	176.42	-0.456			
T_2E_{l2}	198.342	-0.049			
E_{u1}^2	63.383	0.988			
E_{u1}	42.65	-5.371			
$E_{l1}V_{u1}$	9.078	-0.0592			
$E_{u1}E_{l1}$	4.422	0.0678			
			0.287	0.984	0.968

Beyond this point, no term had $F > F$-to-enter so the polynomial was not further expanded and the fitting process was finished. The final polynomial is that of eq. 7 of the manuscript.

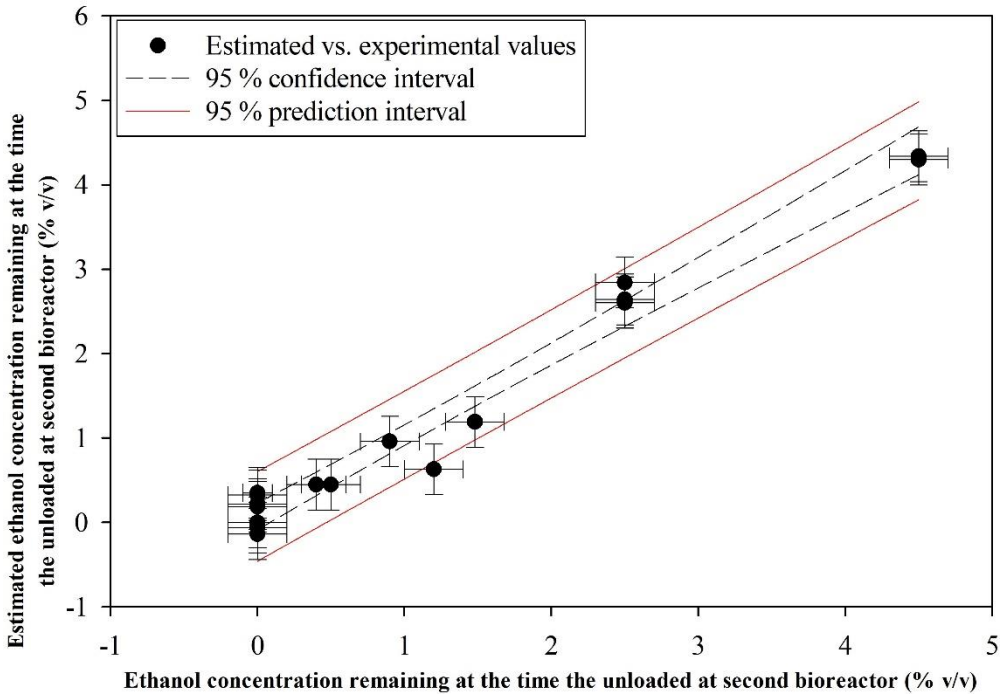

Figure S3.5. Plot of $E_{u2\,est}$ against E_{u2} and curves for the 95% confidence and prediction intervals.

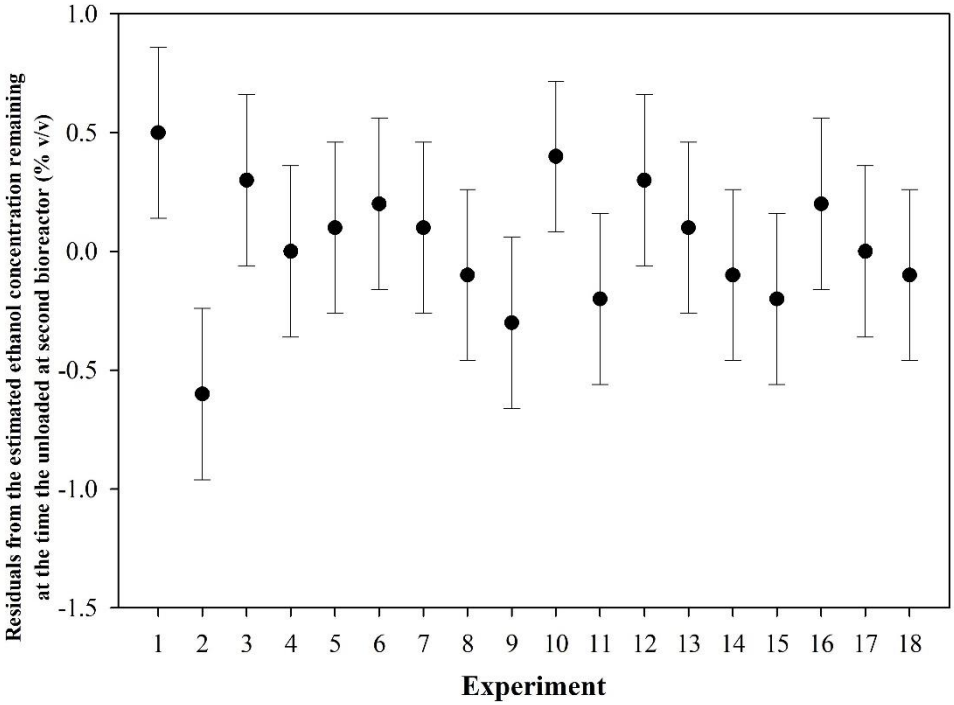

Figure S3.6. Residuals of the fitting of $E_{u2\,est}$ for each experiment.

S3.4. Volume of fermentation medium unloaded from the second reactor

The experimental results of the ANOVA on this variable are shown in Table S3.42.

Table S3.42. Results of the ANOVA on the volume of fermentation medium unloaded from the second reactor.

Source of variability	Degrees of freedom	Variance	*F*	*P-value*
Group	17	10.6	4240.012	<0.001
Sample	144	0.0025		
Total	161			

The *F* value at the 95% confidence level was greater than the corresponding critical point (F_{crit} = 1.623), so there were statistically significant differences between experimental means (*P-value* < 0.001).

The Forward Stepwise Regression method was applied. *F*-to-enter was set at 4 and *F*-to-remove at 3.9. Tables S3.43 to S3.53 show the results of each step. The terms added in each step are shown in successive rows in each table.

Table S3.43. Results of Step 0 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	Coefficient	Standard error
Constant	7.449	
		1.038

Table S3.44. Results of Step 1 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		3.316			
V_{u1}	24.842	0.827			
			0.862	0.569	0.323

Table S3.45. Results of Step 2 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		4.884			
V_{u1}	37.758	0.827			
T_2E_{u1}	28.037	-0.0174			
			0.699	0.751	0.563

Table S3.46. Results of Step 3 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		9.482			
V_{u1}	0.348	-0.134			
T_2E_{u1}	39.824	-0.0685			
$E_{u1}V_{u1}$	23.648	0.32			
			0.582	0.839	0.704

V_{u1} must be removed in the next step.

Table S3.47. Results of Step 4 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		8.834			
T_2E_{u1}	117.561	-0.0631			
$E_{u1}V_{u1}$	78.835	0.286			
			0.578	0.838	0.701

Table S3.48. Results of Step 5 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		7.678			
T_2E_{u1}	186.114	-0.0651			
$E_{u1}V_{u1}$	125.159	0.295			
T_2E_{l2}	26.506	0.0114			
			0.472	0.897	0.805

Table S3.49. Results of Step 6 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		7.732			
T_2E_{u1}	215.749	-0.0963			
$E_{u1}V_{u1}$	157.405	0.484			
T_2E_{l2}	56.335	0.043			
$E_{l2}V_{u1}$	33.476	-0.194			
			0.367	0.940	0.884

Table S3.50. Results of Step 7 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		6.648			
T_2E_{u1}	76.668	-0.0723			
$E_{u1}V_{u1}$	222.462	0.528			
T_2E_{l2}	77.567	0.0442			
$E_{l2}V_{u1}$	49.889	-0.208			
E_{u1}^2	16.285	-0.162			
			0.321	0.956	0.913

Table S3.51. Results of Step 8 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		7.002			
T_2E_{u1}	81.732	-0.0591			
$E_{u1}V_{u1}$	432.552	0.566			
T_2E_{l2}	46.76	0.03			
$E_{l2}V_{u1}$	114.168	-0.244			
E_{u1}^2	67.625	-0.376			
$E_{l2}E_{u1}$	38.587	0.202			
			0.240	0.976	0.952

Table S3.52. Results of Step 9 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		4.639			
T_2E_{u1}	2.596	-0.0227			
$E_{u1}V_{u1}$	240.098	0.665			
T_2E_{l2}	0.137	-0.00472			
$E_{l2}V_{u1}$	77.85	-0.334			
E_{u1}^2	42.361	-0.622			
$E_{l2}E_{u1}$	27.722	0.17			
E_{l2}	8.244	1.593			
			0.224	0.980	0.960

T_2E_{l2} must be removed in the next step.

Table S3.53. Results of Step 10 in the fitting of the volume of fermentation medium unloaded from the second reactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		4.921			
T_2E_{u1}	31.037	-0.0275			
$E_{u1}V_{u1}$	421.63	0.665			
$E_{l2}V_{u1}$	134.502	-0.324			
E_{u1}^2	203.249	-0.59			
$E_{l2}E_{u1}$	29.82	0.172			
E_{l2}	63.235	1.399			
			0.222	0.980	0.960

Beyond this point, no term had $F > F$-to-enter so the polynomial was not further expanded and the fitting process was finished. The final polynomial is that of eq. 8 of the manuscript.

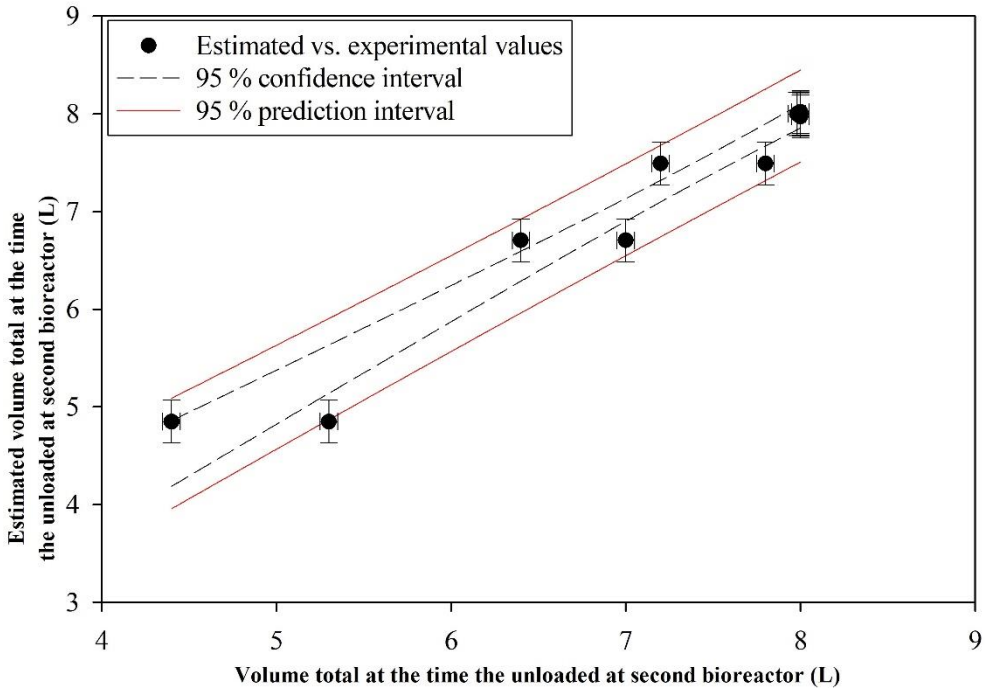

Figure S3.7. Plot of $V_{u2\,est}$ against V_{u2} and curves for the 95% confidence and prediction intervals.

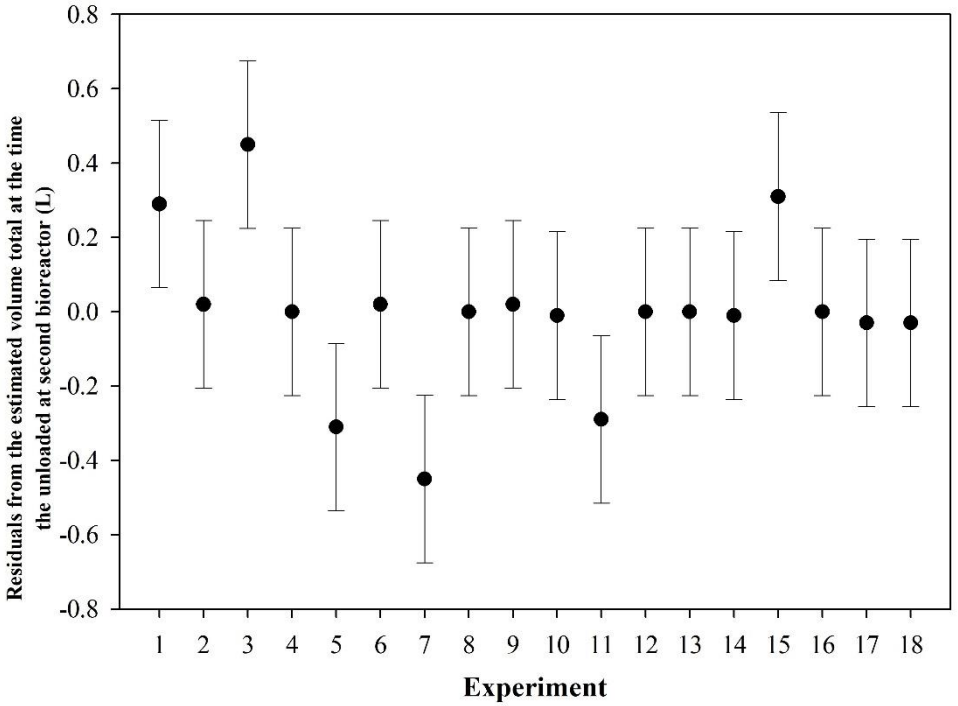

Figure S3.8. Residuals of the fitting of $V_{u2\,est}$ for each experiment.

S3.5. Total cycle duration

The experimental results of the ANOVA on this variable are shown in Table S3.54.

Table S3.54. Results of the ANOVA on the total cycle duration.

Source of variability	Degrees of freedom	Variance	*F*	*P-value*

Group	17	931.949	1456.644	<0.001
Sample	144	0.64		
Total	161			

The F value at the 95% confidence level was greater than the corresponding critical point (F_{crit} = 1.623), so there were statistically significant differences between experimental means (*P-value* < 0.001).

The Forward Stepwise Regression method was applied. F-to-enter was set at 4 and F-to-remove at 3.9. Tables S3.55 to S3.60 show the results of each step. The terms added in each step are shown in successive rows in each table.

Table S3.55. Results of Step 0 in the fitting of the total cycle duration.

Terms added	Coefficient	Standard error
Constant	35.228	
		1.381

Table S3.56. Results of Step 1 in the fitting of the total cycle duration.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		6.512			
V_{u1}^2	87.643	1.126			
			6.252	0.792	0.628

Table S3.57. Results of Step 2 in the fitting of the total cycle duration.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		18.78			
V_{u1}^2	192.226	1.126			
$E_{u1}E_{l1}$	63.05	-0.818			
			4.222	0.913	0.833

Table S3.58. Results of Step 3 in the fitting of the total cycle duration.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		323.69			
V_{u1}^2	23.922	13.556			
$E_{u1}E_{cul1}$	86.694	-0.818			
V_{u1}	20.125	-124.372			
			3.600	0.939	0.881

Table S3.59. Results of Step 4 in the fitting of the total cycle duration.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		356.503			
V_{u1}^2	36.313	13.556			
$E_{u1}E_{l1}$	131.599	-0.818			
V_{u1}	30.55	-124.372			
T_1	26.898	-1.094			
			2.922	0.961	0.923

Table S3.60. Results of Step 5 in the fitting of the total cycle duration.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		518.591			
V_{u1}^2	50.123	13.556			
$E_{u1}E_{l1}$	196.913	-0.864			
V_{u1}	58.441	-156.652			

T_1	27.82	-6.474			
T_1V_{u1}	19.634	1.076			
			2.487	0.972	0.946

Beyond this point, no term had $F > F$-to-enter so the polynomial was not further expanded and the fitting process was finished. The final polynomial is that of eq. 9 of the manuscript.

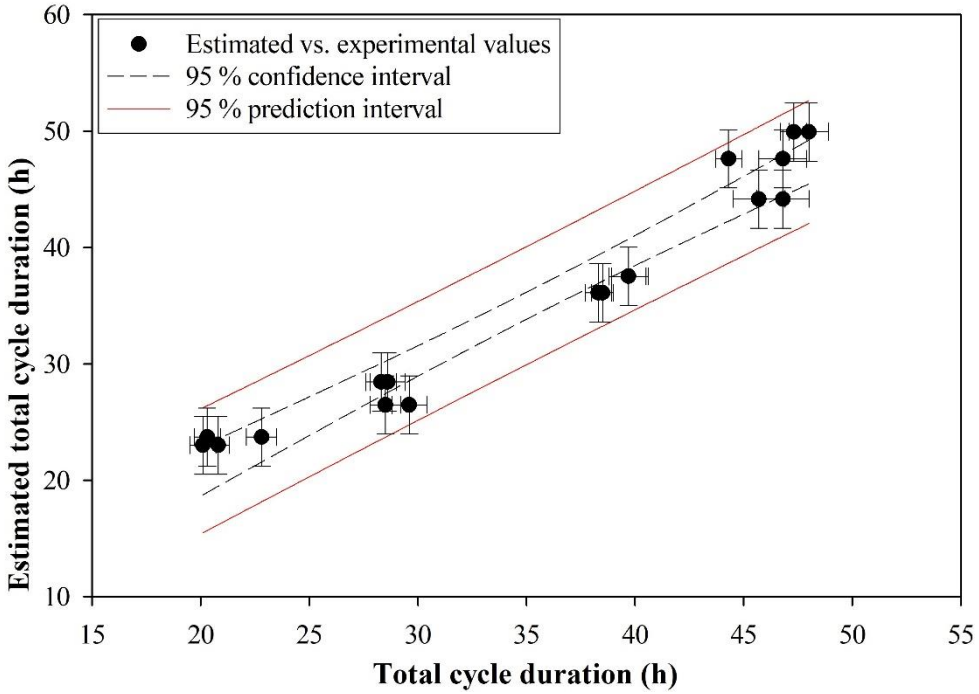

Figure S3.9. Plot of $t_{cycle\ est}$ against t_{cycle} and curves for the 95% confidence and prediction intervals

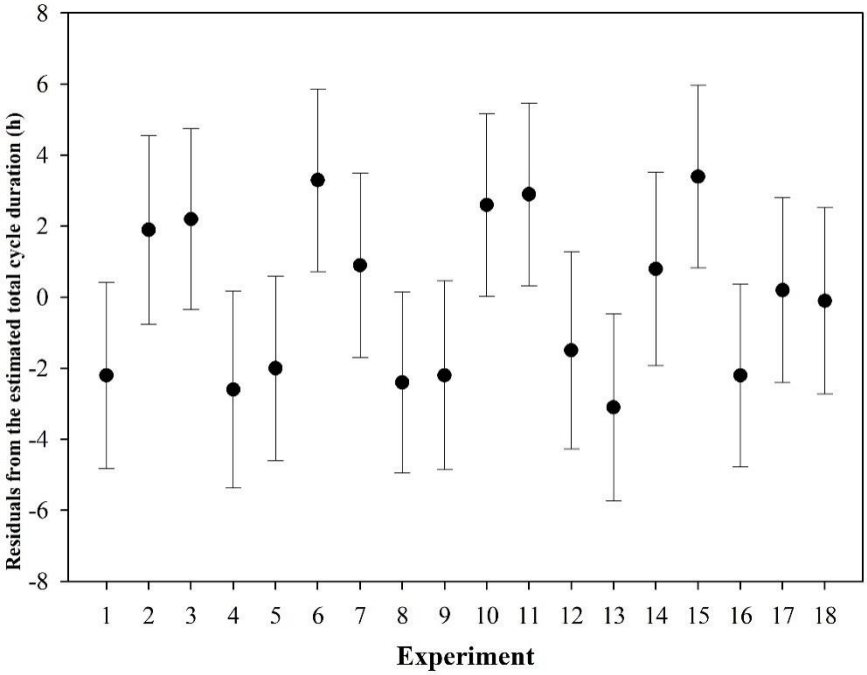

Figure S3.10. Residuals of the fitting of $t_{cycle\ est}$ for each experiment.

S3.6. Mean overall volume in the two-bioreactor system

The experimental results of the ANOVA on this variable are shown in Table S3.61.

Table S3.61. Results of the ANOVA on the mean overall volume in the two-bioreactor system.

Source of variability	Degrees of freedom	Variance	*F*	*P-value*
Group	17	15.027	105.912	<0.001
Sample	144	0.142		
Total	161			

The F value at the 95% confidence level was greater than the corresponding critical point (F_{crit} = 1.623), so there were statistically significant differences between experimental means (*P-value* < 0.001).

The Forward Stepwise Regression method was applied. F-to-enter was set at 4 and F-to-remove at 3.9. Tables S3.62 to S3.74 show the results of each step. The terms added in each step are shown in successive rows in each table.

Table S3.62. Results of Step 0 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	Coefficient	Standard error
Constant	13.197	
		1.255

Table S3.63. Results of Step 1 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		14.591			
E_{u1}^2	36.529	-0.141			
			0.971	0.642	0.413

Table S3.64. Results of Step 2 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		11.746			
E_{u1}^2	72.053	-0.141			
$E_{l1}V_{u1}$	51.568	0.114			
			0.691	0.841	0.708

Table S3.65. Results of Step 3 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		10.179			
E_{u1}^2	121.374	-0.141			
$E_{l1}V_{u1}$	86.867	0.114			
T_2E_{l2}	35.911	0.0149			
			0.533	0.911	0.830

Table S3.66. Results of Step 4 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		4.46			
E_{u1}^2	16.007	-0.841			
$E_{l1}V_{u1}$	104.463	0.114			
T_2E_{l2}	43.184	0.0149			
E_{u1}	11.128	4.215			
			0.486	0.928	0.861

Table S3.67. Results of Step 5 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		4.918			
E_{u1}^2	17.636	-0.841			

$E_{l1}V_{u1}$	55.749	0.096			
T_2E_{l2}	46.717	0.0148			
E_{u1}	9.755	3.797			
$E_{u1}V_{u1}$	5.988	0.0835			
			0.463	0.936	0.877

Table S3.68. Results of Step 6 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		10.021			
E_{u1}^2	24.054	-0.841			
$E_{l1}V_{u1}$	89.676	0.107			
T_2E_{l2}	59.454	0.0143			
E_{u1}	4.305	2.281			
$E_{u1}V_{u1}$	25.64	0.387			
V_{u1}	18.466	-1.066			
			0.396	0.955	0.912

Table S3.69. Results of Step 7 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		9.88			
E_{u1}^2	26.752	-0.841			
$E_{l1}V_{u1}$	99.736	0.107			
T_2E_{l2}	71.916	0.0153			
E_{u1}	7.528	2.953			
$E_{u1}V_{u1}$	28.13	0.384			
V_{u1}	20.235	-1.058			
T_2E_{u1}	6.272	-0.022			
			0.376	0.960	0.922

Table S3.70. Results of Step 8 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		9.189			
E_{u1}^2	29.611	-0.841			
$E_{l1}V_{u1}$	32.551	0.179			
T_2E_{l2}	19.624	0.0328			
E_{u1}	12.147	3.739			
$E_{u1}V_{u1}$	22.285	0.337			
V_{u1}	28.098	-1.28			
T_2E_{u1}	12.859	-0.0404			
$E_{l2}E_{l1}$	5.917	-0.106			
			0.357	0.965	0.931

Table S3.71. Results of Step 9 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		5.664			
E_{u1}^2	33.801	-0.841			
$E_{l1}V_{u1}$	1.182	0.0582			
T_2E_{l2}	25.945	0.0357			
E_{u1}	20.151	4.899			
$E_{u1}V_{u1}$	24.231	0.33			
V_{u1}	4.026	-0.65			

T_2E_{u1}	20.11	-0.0777			
$E_{l2}E_{l1}$	9.095	-0.124			
T_2E_{l1}	7.366	0.0229			
			0.334	0.970	0.941

$E_{l1}V_{u1}$ must be removed in the next step.

Table S3.72. Results of Step 10 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	***F***	**Coefficient**	**Standard error**	***R***	***R*²**
Constant		4.635			
E_{u1}^2	33.665	-0.841			
T_2E_{l2}	25.418	0.0329			
E_{u1}	22.641	5.116			
$E_{u1}V_{u1}$	25.531	0.337			
V_{u1}	3.294	-0.382			
T_2E_{u1}	30.906	-0.0862			
$E_{l2}E_{l1}$	7.883	-0.108			
T_2E_{l1}	43.166	0.0305			
			0.335	0.969	0.940

V_{u1} must be removed in the next step.

Table S3.73. Results of Step 11 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	***F***	**Coefficient**	**Standard error**	***R***	***R*²**
Constant		2.426			
E_{u1}^2	32.065	-0.841			
T_2E_{l2}	30.616	0.0358			
E_{u1}	33.394	5.87			
$E_{u1}V_{u1}$	112.22	0.222			
T_2E_{u1}	35.198	-0.0922			
$E_{l2}E_{l1}$	10.64	-0.124			
T_2E_{l1}	48.891	0.0324			
			0.343	0.967	0.935

Table S3.74. Results of Step 12 in the fitting of the mean overall volume in the two-bioreactor system.

Terms added	***F***	**Coefficient**	**Standard error**	***R***	***R*²**
Constant		4.306			
E_{u1}^2	34.441	-0.841			
T_2E_{l2}	32.872	0.0358			
E_{u1}	17.949	4.739			
$E_{u1}V_{u1}$	33.616	0.336			
T_2E_{u1}	17.742	-0.0735			
$E_{l2}E_{l1}$	11.523	-0.125			
T_2E_{l1}	53.134	0.0326			
T_2V_{u1}	4.408	-0.0127			
			0.331	0.970	0.941

Beyond this point, no term had $F > F$-to-enter so the polynomial was not further expanded and the fitting process was finished. The final polynomial is that of eq. 10 of the manuscript.

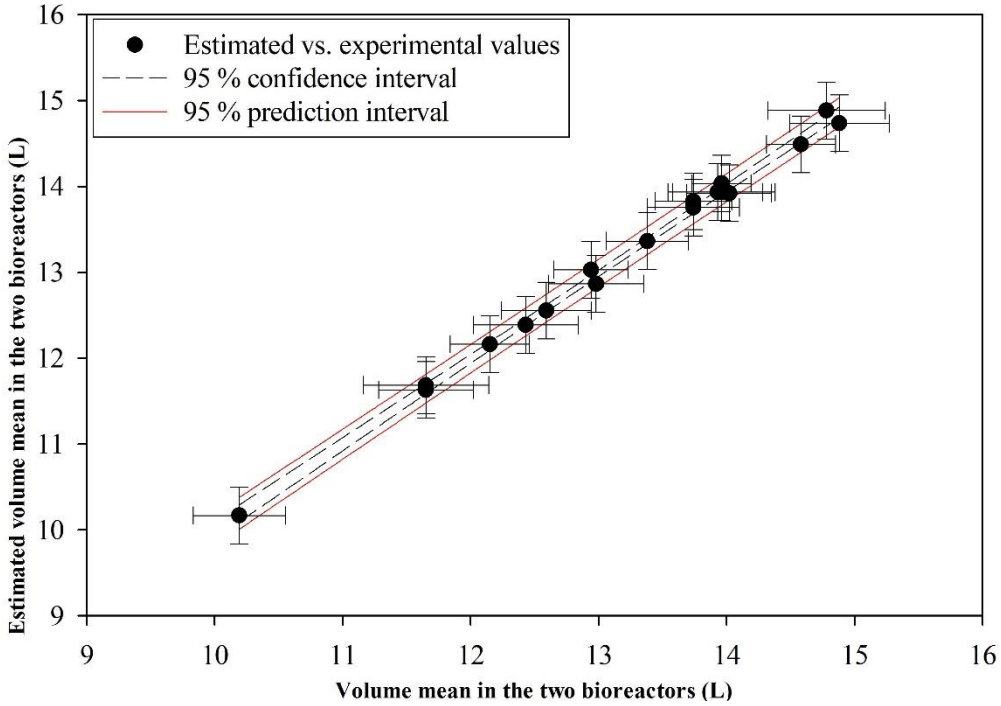

Figure S3.11. Plot of $V_{m\,est}$ against V_m and curves for the 95% confidence and prediction intervals.

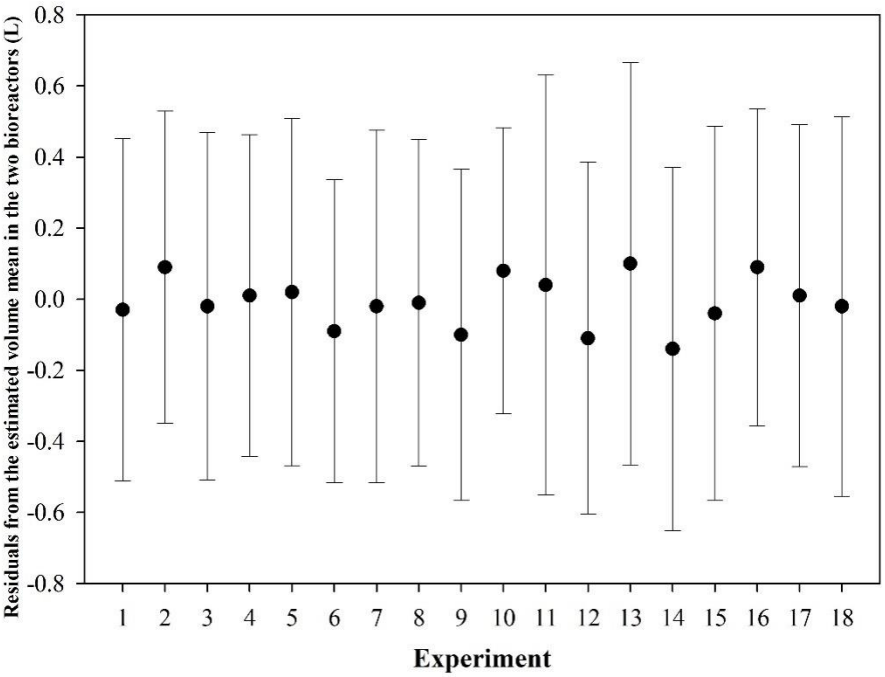

Figure S3.12. Residuals of the fitting of $V_{m\,est}$ for each experiment

S3.7. Mean ethanol concentration in the first bioreactor

The experimental results of the ANOVA on this variable are shown in Table S3.75.

Table S3.75. Results of the ANOVA on the mean ethanol concentration in the first bioreactor.

Source of variability	Degrees of freedom	Variance	*F*	*P-value*
Group	17	3.957	89.301	<0.001
Sample	144	0.0443		
Total	161			

The F value at the 95% confidence level was greater than the corresponding critical point (F_{crit} = 1.623), so there were statistically significant differences between experimental means (*P-value* < 0.001).

The Forward Stepwise Regression method was applied. F-to-enter was set at 4 and F-to-remove at 3.9. Tables S3.76 to S3.81 show the results of each step. The terms added in each step are shown in successive rows in each table.

Table S3.76. Results of Step 0 in the fitting of the mean ethanol concentration in the first bioreactor.

Terms added	Coefficient	Standard error
Constant	4.494	
		0.713

Table S3.77. Results of Step 1 in the fitting of the mean ethanol concentration in the first bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		1.401			
E_{l1}	111.747	0.619			
			0.406	0.826	0.682

Table S3.78. Results of Step 2 in the fitting of the mean ethanol concentration in the first bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		1.401			
E_{l1}	111.404	0.415			
$E_{u1}E_{l1}$	103.843	0.0678			
			0.235	0.946	0.895

Table S3.79. Results of Step 3 in the fitting of the mean ethanol concentration in the first bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		2.372			
E_{l1}	0.689	0.0755			
$E_{u1}E_{l1}$	39.887	0.181			
E_{u1}^2	16.287	-0.0982			
			0.206	0.960	0.921

E_{l1} must be removed in the next step.

Table S3.80. Results of Step 4 in the fitting of the mean ethanol concentration in the first bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		2.597			
$E_{u1}E_{l1}$	474.589	0.204			
E_{u1}^2	161.378	-0.117			
			0.206	0.959	0.920

Table S3.81. Results of Step 5 in the fitting of the mean ethanol concentration in the first bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		2.312			
$E_{u1}E_{l1}$	174.929	0.175			
E_{u1}^2	61.047	-0.0932			
$E_{l1}V_{u1}$	8.218	0.0191			
			0.192	0.965	0.931

Beyond this point, no term had F > F-to-enter so the polynomial was not further expanded and the fitting process was finished. The final polynomial is that of eq. 11 of the manuscript.

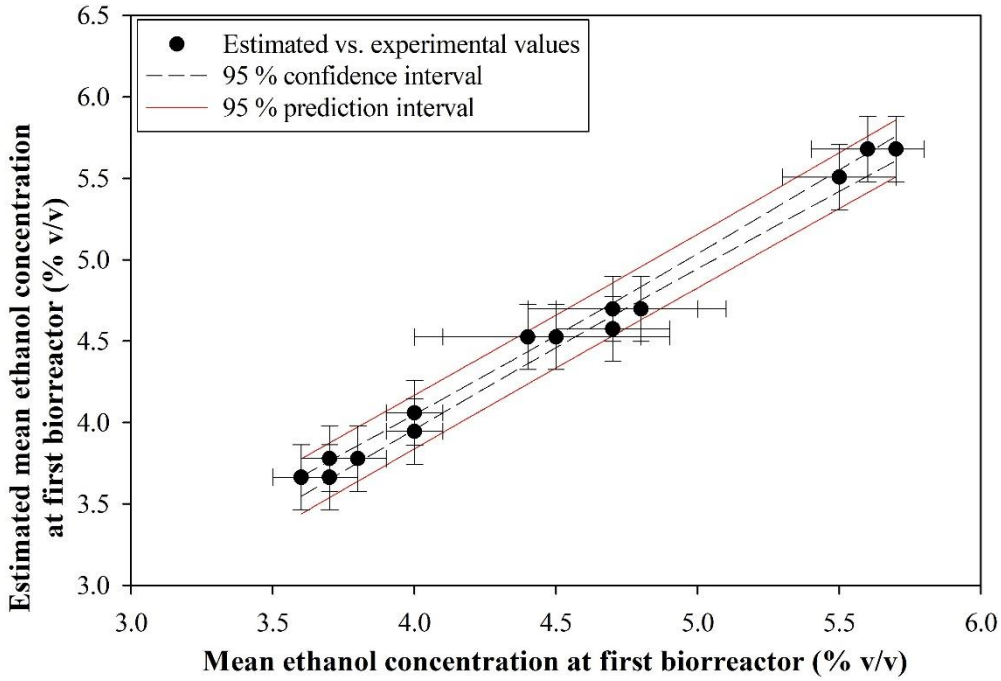

Figure S3.13. Plot of $EtOH_{m1\,est}$ against $EtOH_{m1}$ and curves for the 95% confidence and prediction intervals.

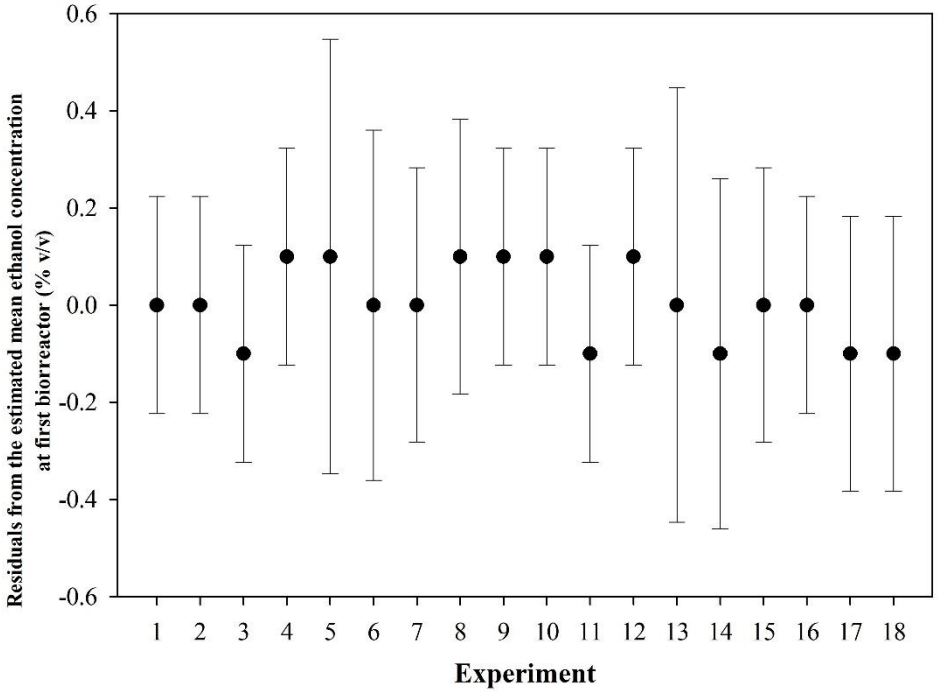

Figure S3.14. Residuals of the fitting of $EtOH_{m1\,est}$ for each experiment.

S3.8. Mean ethanol concentration in the second bioreactor

The experimental results of the ANOVA on this variable are shown in Table S3.82.

Table S3.82. Results of the ANOVA on the mean ethanol concentration in the second bioreactor.

Source of variability	Degrees of freedom	Variance	*F*	*P-value*
Group	17	5.175	90.883	<0.001

Sample	144	0.0569
Total	161	

The F value at the 95% confidence level was greater than the corresponding critical point (F_{crit} = 1.623), so there were statistically significant differences between experimental means (*P-value* < 0.001).

The Forward Stepwise Regression method was applied. F-to-enter was set at 4 and F-to-remove at 3.9. Tables S3.83 to S3.88 show the results of each step. The terms added in each step are shown in successive rows in each table.

Table S3.83. Results of Step 0 in the fitting of the mean ethanol concentration in the second bioreactor.

Terms added	Coefficient	Standard error
Constant	2.944	
		0.777

Table S3.84. Results of Step 1 in the fitting of the mean ethanol concentration in the second bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		5.728			
T_2V_{u1}	23.855	-0.0186			
			0.649	0.561	0.314

Table S3.85. Results of Step 2 in the fitting of the mean ethanol concentration in the second bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		4.788			
T_2V_{u1}	41.308	-0.0188			
$E_{l2}E_{u1}$	36.878	0.0928			
			0.500	0.776	0.602

Table S3.86. Results of Step 3 in the fitting of the mean ethanol concentration in the second bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		4.44			
T_2V_{u1}	20.072	-0.0135			
$E_{l2}E_{u1}$	52.497	0.143			
$E_{u1}V_{u1}$	12.67	-0.0646			
			0.451	0.826	0.683

Table S3.87. Results of Step 4 in the fitting of the mean ethanol concentration in the second bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		4.638			
T_2V_{u1}	2.297	-0.00619			
$E_{l2}E_{u1}$	28.734	0.253			
$E_{u1}V_{u1}$	16.654	-0.139			
T_2E_{l2}	6.391	-0.0127			
			0.428	0.848	0.719

T_2V_{u1} must be removed in the next step.

Table S3.88. Results of Step 5 in the fitting of the mean ethanol concentration in the second bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		4.327			
$E_{l2}E_{u1}$	93.41	0.306			
$E_{u1}V_{u1}$	71.239	-0.179			
T_2E_{l2}	25.665	-0.0182			

	0.434	0.840	0.706

Beyond this point, no term had $F > F$-to-enter so the polynomial was not further expanded and the fitting process was finished. The final polynomial is that of eq. 12 of the manuscript.

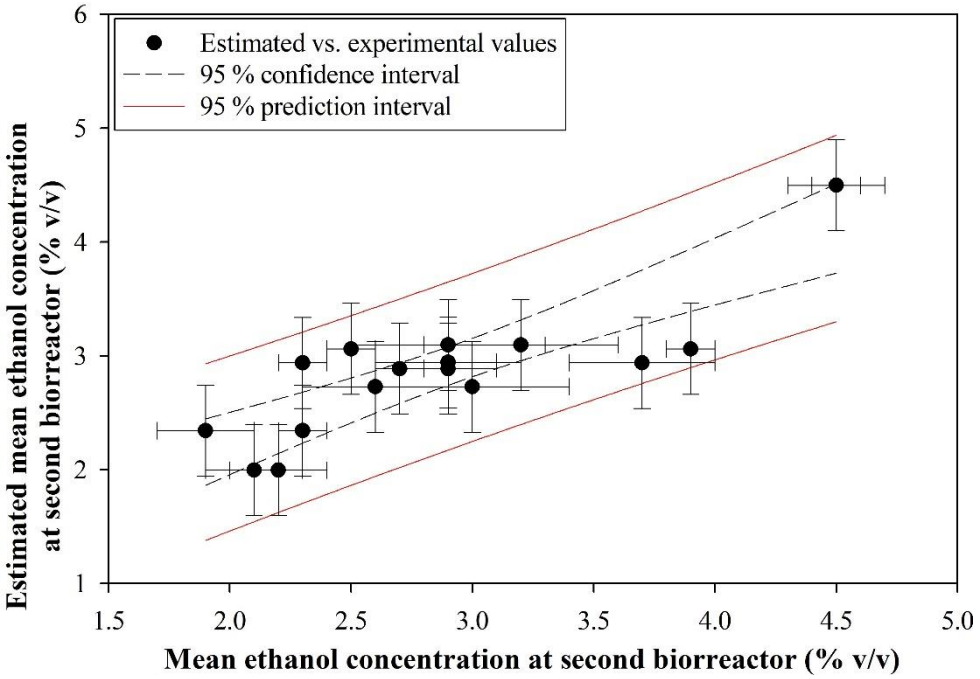

Figure S3.15. Plot of $EtOH_{m2\,est}$ against $EtOH_{m2}$ and curves for the 95% confidence and prediction intervals.

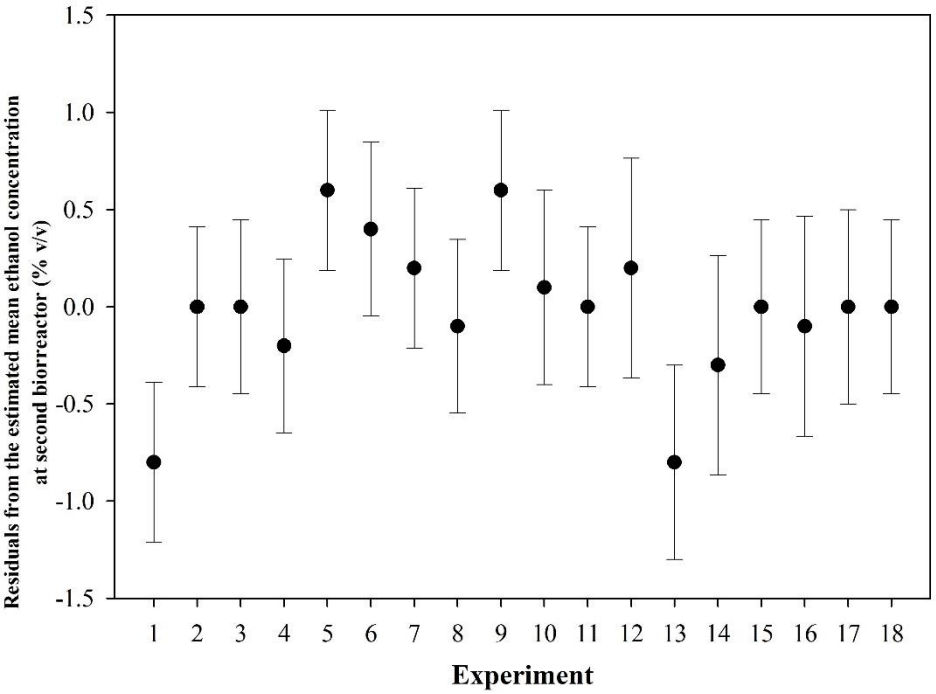

Figure S3.16. Residuals of the fitting of $EtOH_{m2\,est}$ for each experiment.

S3.9. Mean acetic acid concentration in the first bioreactor

The experimental results of the ANOVA on this variable are shown in Table S3.89.

Table S3.89. Results of the ANOVA on the mean acetic acid concentration in the first bioreactor.

Source of variability	Degrees of freedom	Variance	*F*	*P-value*
Group	17	3.957	89.301	<0.001
Sample	144	0.0443		
Total	161			

The F value at the 95% confidence level was greater than the corresponding critical point (F_{crit} = 1.623), so there were statistically significant differences between experimental means (*P-value* < 0.001).

The Forward Stepwise Regression method was applied. F-to-enter was set at 4 and F-to-remove at 3.9. Tables S3.90 to S3.95 show the results of each step. The terms added in each step are shown in successive rows in each table.

Table S3.90. Results of Step 0 in the fitting of the mean acetic acid concentration in the first bioreactor.

Terms added	Coefficient	Standard error
Constant	7.006	
		0.713

Table S3.91. Results of Step 1 in the fitting of the mean acetic acid concentration in the first bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		10.099			
E_{l1}	111.747	-0.619			
			0.406	0.826	0.682

Table S3.92. Results of Step 2 in the fitting of the mean acetic acid concentration in the first bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		10.099			
E_{l1}	111.404	-0.415			
$E_{u1}E_{l1}$	103.843	-0.0678			
			0.235	0.946	0.895

Table S3.93. Results of Step 3 in the fitting of the mean acetic acid concentration in the first bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		9.128			
E_{l1}	0.689	-0.0755			
$E_{u1}E_{l1}$	39.887	-0.181			
E_{u1}^2	16.287	0.0982			
			0.206	0.960	0.921

E_{l1} must be removed in the next step.

Table S3.94. Results of Step 4 in the fitting of the mean acetic acid concentration in the first bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		8.903			
$E_{u1}E_{l1}$	474.589	-0.204			
E_{u1}^2	161.378	0.117			
			0.206	0.959	0.920

Table S3.95. Results of Step 5 in the fitting of the mean acetic acid concentration in the first bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		9.188			
$E_{u1}E_{l1}$	174.929	-0.175			

E_{u1}^2	61.047	0.0932			
$E_{l1}V_{u1}$	8.218	-0.0191			
			0.192	0.965	0.931

Beyond this point, no term had $F > F$-to-enter so the polynomial was not further expanded and the fitting process was finished. The final polynomial is that of eq. 13 of the manuscript.

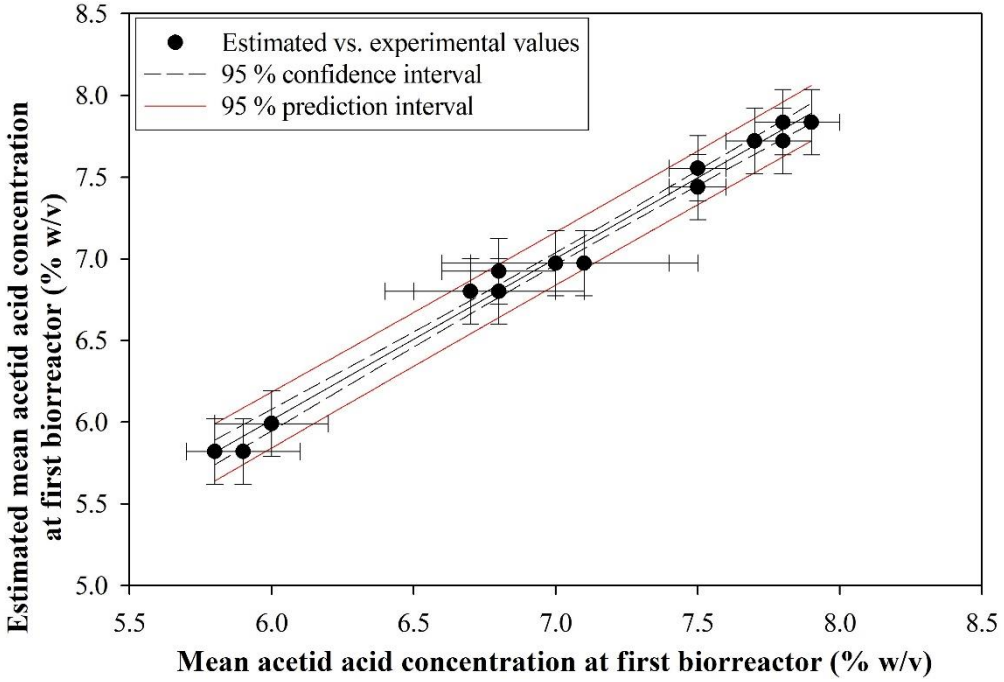

Figure S3.17. Plot of $HAc_{m1\,est}$ against HAc_{m1} and curves for the 95% confidence and prediction intervals.

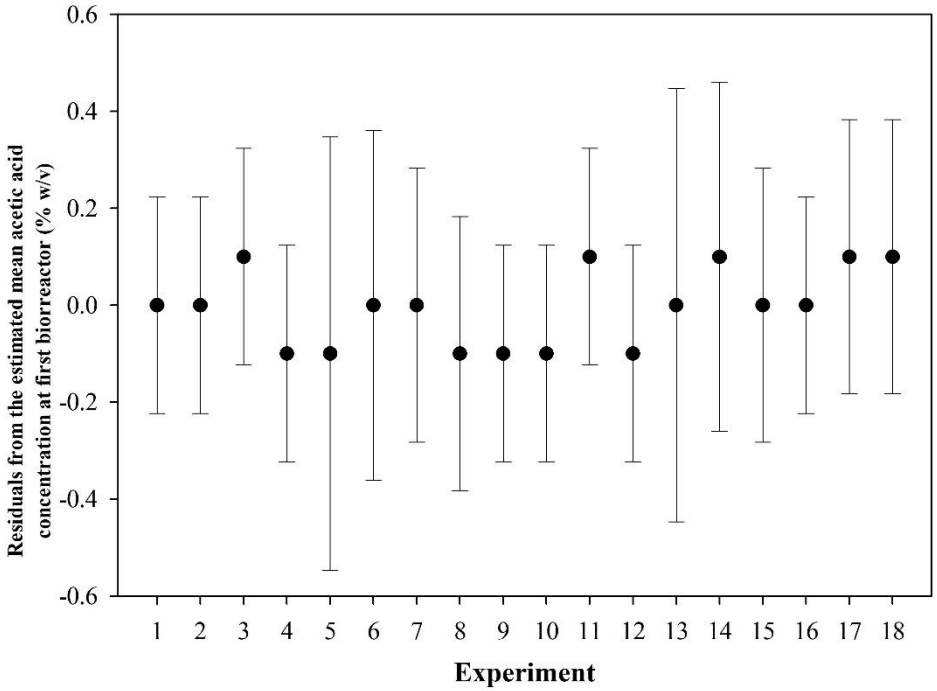

Figure S3.18. Residuals of the fitting of $HAc_{m1\,est}$ for each experiment.

S3.10. Mean acetic acid concentration in the second bioreactor

The experimental results of the ANOVA on this variable are shown in Table S3.96.

Table S3.96. Results of the ANOVA on the mean acetic acid concentration in the second bioreactor.

Source of variability	Degrees of freedom	Variance	*F*	*P-value*
Group	17	5.198	91.279	<0.001
Sample	144	0.0569		
Total	161			

The F value at the 95% confidence level was greater than the corresponding critical point (F_{crit} = 1.623), so there were statistically significant differences between experimental means (*P-value* < 0.001).

The Forward Stepwise Regression method was applied. F-to-enter was set at 4 and F-to-remove at 3.9. Tables S3.97 to S3.102 show the results of each step. The terms added in each step are shown in successive rows in each table.

Table S3.97. Results of Step 0 in the fitting of the mean acetic acid concentration in the second bioreactor.

Terms added	Coefficient	Standard error
Constant	8.55	
		0.779

Table S3.98. Results of Step 1 in the fitting of the mean acetic acid concentration in the second bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		5.818			
T_2V_{u1}	22.397	0.0182			
			0.658	0.549	0.301

Table S3.99. Results of Step 2 in the fitting of the mean acetic acid concentration in the second bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		6.78			
T_2V_{u1}	39.379	0.0184			
$E_{l2}E_{u1}$	38.136	-0.0949			
			0.502	0.775	0.600

Table S3.100. Results of Step 3 in the fitting of the mean acetic acid concentration in the second bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		7.13			
T_2V_{u1}	18.781	0.0132			
$E_{l2}E_{u1}$	53.604	-0.146			
$E_{u1}V_{u1}$	12.646	0.0649			
			0.453	0.825	0.681

Table S3.101. Results of Step 4 in the fitting of the mean acetic acid concentration in the second bioreactor.

Terms added	*F*	Coefficient	Standard error	*R*	R^2
Constant		6.933			
T_2V_{u1}	2.029	0.00586			
$E_{l2}E_{u1}$	28.733	-0.254			

$E_{u1}V_{u1}$	16.406	0.138			
T_2E_{l2}	6.239	0.0127			
			0.431	0.847	0.717

T_2V_{u1} must be removed in the next step.

Table S3.102. Results of Step 5 in the fitting of the mean acetic acid concentration in the second bioreactor.

Terms added	***F***	**Coefficient**	**Standard error**	***R***	***R*²**
Constant		7.227			
$E_{l2}E_{u1}$	91.936	-0.305			
$E_{u1}V_{u1}$	68.862	0.177			
T_2E_{l2}	24.457	0.0178			
			0.436	0.840	0.706

Beyond this point, no term had $F > F$-to-enter so the polynomial was not further expanded and the fitting process was finished. The final polynomial is that of eq. 14 of the manuscript.

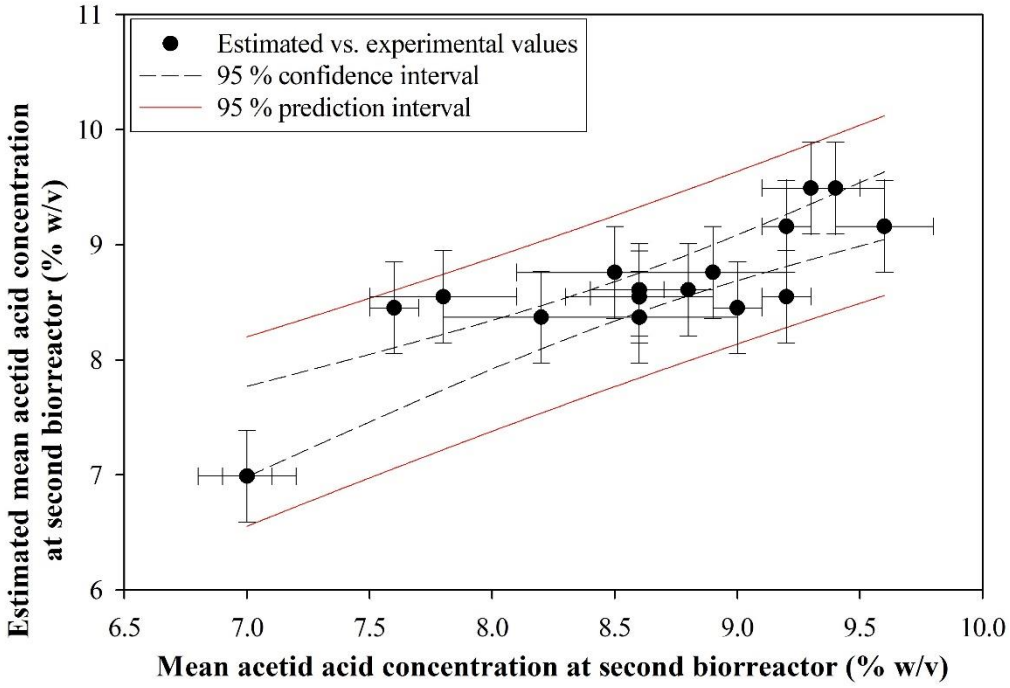

Figure S3.19. Plot of $HAc_{m2\,est}$ against HAc_{m2} and curves for the 95% confidence and prediction intervals.

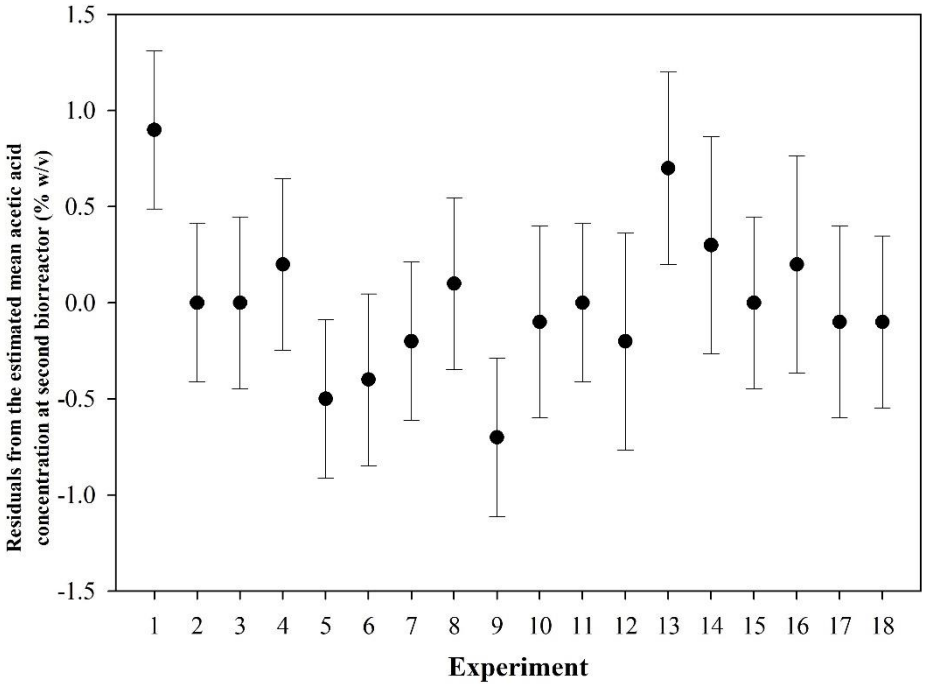

Figure S3.20. Residuals of the fitting of $HAc_{m2\ est}$ for each experiment.

Abbreviations

E_{l1}: ethanol concentration while the first reactor was loaded (% v/v).
E_{l2}: ethanol concentration while the second reactor was loaded (% v/v).
$EtOH_{m1}$: mean ethanol concentration in the first reactor during a cycle (% v/v).
$EtOH_{m1\ est}$: estimated mean ethanol concentration in the first reactor during a cycle (% v/v).
$EtOH_{m2}$: mean ethanol concentration in the second reactor during a cycle (% v/v).
$EtOH_{m2\ est}$: estimated mean ethanol concentration in the second reactor during a cycle (% v/v).
E_{u1}: ethanol concentration at the time the first reactor was unloaded (% v/v).
E_{u2}: ethanol concentration at the time the second reactor was unloaded (% v/v).
$E_{u2\ est}$: estimated ethanol concentration at the time the second reactor was unloaded (% v/v).
HAc_{m1}: mean acetic acid concentration in the first reactor during a cycle (% w/v).
$HAc_{m1\ est}$: estimated mean acetic acid concentration in the first reactor during a cycle (% w/v).
HAc_{m2}: mean acetic acid concentration in the second reactor during a cycle (% w/v).
$HAc_{m2\ est}$: estimated mean acetic acid concentration in the second reactor during a cycle (% w/v).
P_m: total production of acetic acid in the two-reactor system (g acetic acid·h^{-1}).
$P_{m\ est}$: estimated total production of acetic acid in the two-reactor system (g acetic acid·h^{-1}).
$(r_A)_{global}$: mean overall rate of acetic acid formation in the two bioreactors (% w/v ·h^{-1}).
$(r_A)_{global\ est}$: estimated mean overall rate of acetic acid formation in the two bioreactors (% w/v ·h^{-1}).
T_1: temperature in the first reactor (ºC).
T_2: temperature in the second reactor (ºC).
t_{cycle}: total cycle duration (h).
$t_{cycle\ est}$: estimated total cycle duration (h).
V_m: mean overall volume in the two reactors during a cycle (L).
$V_{m\ est}$: estimated mean overall volume in the two reactors during a cycle (L).
V_{u1}: volume unloaded from the first reactor (L).
V_{u2}: volume unloaded from the second reactor (L).
$V_{u2\ est}$: estimated volume unloaded from the second reactor (L).