

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

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The primary aim of this work was to model the two serially arranged bioreactors process for wine vinegar production. This required careful development of an experimental design involving the experiments of Section 3.1 (specifically, those in Tables 2–4). The results provided by the 18 randomly conducted experiments encompassed by the fractional factorial design with central points are described below.

**Table S2.1.** Operating conditions used in each experiment of the proposed design.

Exp. no.	$V_{u1}$	$E_{u1}$	$E_{l1}$	$T_1$	$E_{l2}$	$T_2$
1	4.25	2.0	4.0	28	2.5	28
2	5.75	2.0	4.0	32	2.5	32
3	4.25	4.0	4.0	32	2.5	32
4	5.75	4.0	4.0	28	2.5	28
5	4.25	2.0	6.0	32	2.5	28
6	5.75	2.0	6.0	28	2.5	32
7	4.25	4.0	6.0	28	2.5	32
8	5.75	4.0	6.0	32	2.5	28
9	4.25	2.0	4.0	28	4.5	32
10	5.75	2.0	4.0	32	4.5	28
11	4.25	4.0	4.0	32	4.5	28
12	5.75	4.0	4.0	28	4.5	32
13	4.25	2.0	6.0	32	4.5	32
14	5.75	2.0	6.0	28	4.5	28
15	4.25	4.0	6.0	28	4.5	28
16	5.75	4.0	6.0	32	4.5	32
17	5.00	3.0	5.0	30	3.5	30
18	5.00	3.0	5.0	30	3.5	30

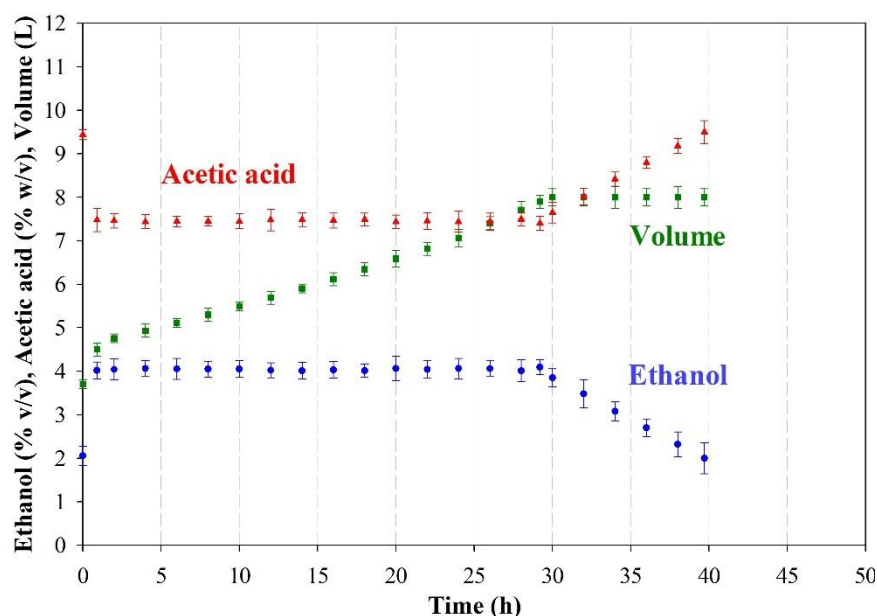
## S2.1. Results of Experiment 1

The operating conditions used in this experiment were as follows:

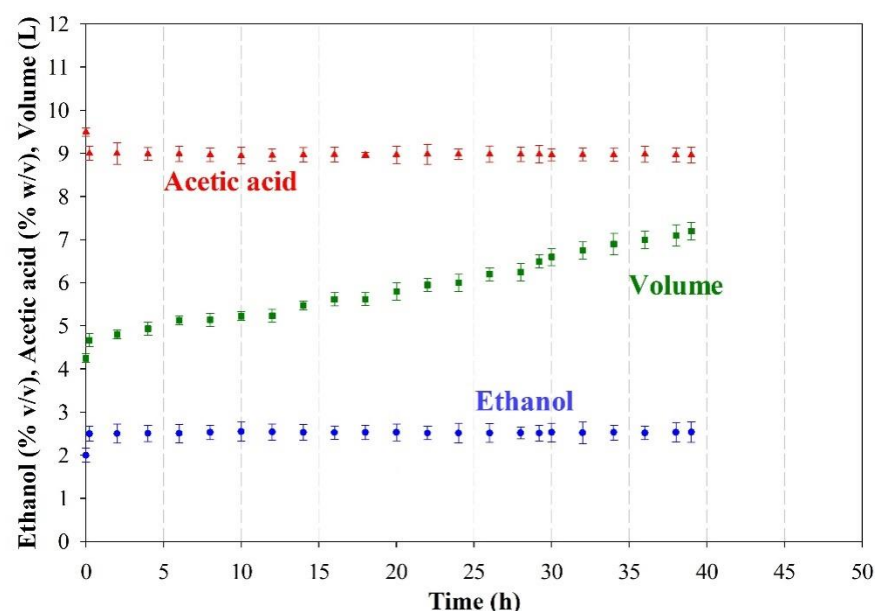
- Volume unloaded from the first bioreactor: 4.25 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 2.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 4.0 % (v/v).

- Temperature in the first bioreactor: 28 °C.
- Ethanol concentration during loading of the second bioreactor: 2.5 % (v/v).
- Temperature in the second bioreactor: 28 °C.

Figures S2.1 and S2.2 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of five replications with their standard deviations.



**Figure S2.1.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 1.



**Figure S2.2.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 1.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.2 shows the values for Experiment 1.

**Table S2.2.** Experimental results obtained in Experiment 1.

Variable	Value
Total cycle duration (h)	$39.7 \pm 0.8$
Duration of the fast loading stage in the first bioreactor (h)	$0.9 \pm 0.1$
Duration of the slow loading stage in the first bioreactor (h)	$28.3 \pm 0.6$
Duration of the depletion stage in the first bioreactor (h)	$10.5 \pm 1.0$
Duration of the fast loading stage in the second bioreactor (h)	$0.2 \pm 0.1$
Duration of the slow loading stage in the second bioreactor (h)	$39.4 \pm 1.2$
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	$0.023 \pm 0.003$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$0.713 \pm 0.021$
Time fraction of the depletion stage in the first bioreactor over the cycle duration	$0.264 \pm 0.026$
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	$0.006 \pm 0.001$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.994 \pm 0.043$
Mean volume in the fast loading stage in the first bioreactor (L)	$4.21 \pm 0.05$
Mean volume in the slow loading stage in the first bioreactor (L)	$6.35 \pm 0.05$
Mean volume in the depletion stage in the first bioreactor (L)	$8.02 \pm 0.05$
Mean volume in the first bioreactor (L)	$6.74 \pm 0.25$
Mean volume in the fast loading stage in the second bioreactor (L)	$4.38 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$5.86 \pm 0.05$
Mean volume in the second bioreactor (L)	$5.85 \pm 0.25$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$12.59 \pm 0.35$
Volume of fermentation medium unloaded from the second reactor (L)	$7.2 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$2.3 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$3.7 \pm 0.1$
Mean ethanol concentration in the second bioreactor (% v/v)	$3.9 \pm 0.1$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$9.4 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$9.2 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$7.8 \pm 0.1$

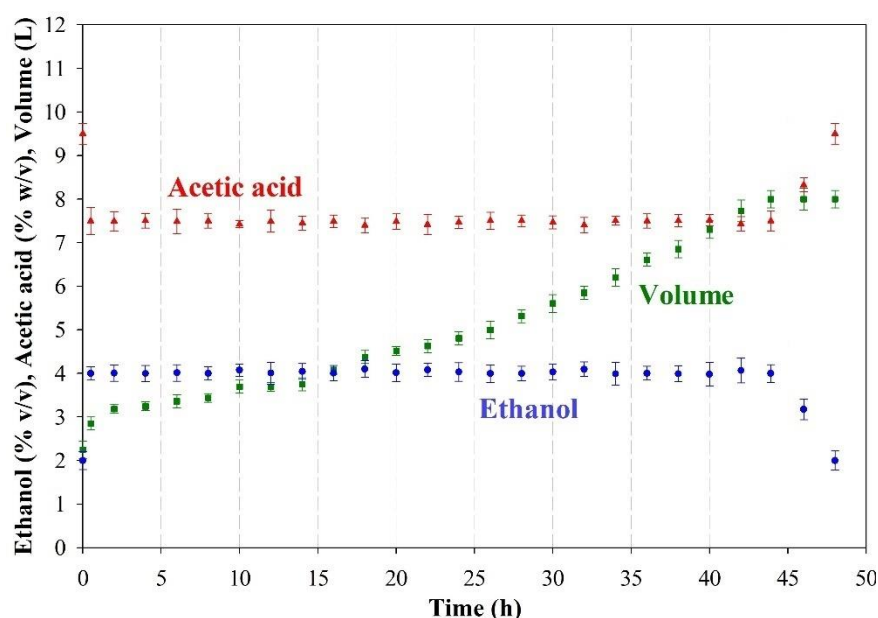
Mean acetic acid concentration in the second bioreactor (% w/v)	$7.6 \pm 0.1$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.15 \pm 0.01$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.11 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.13 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$10.1 \pm 0.3$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$6.6 \pm 0.5$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$16.7 \pm 0.5$

## S2.2. Results of Experiment 2

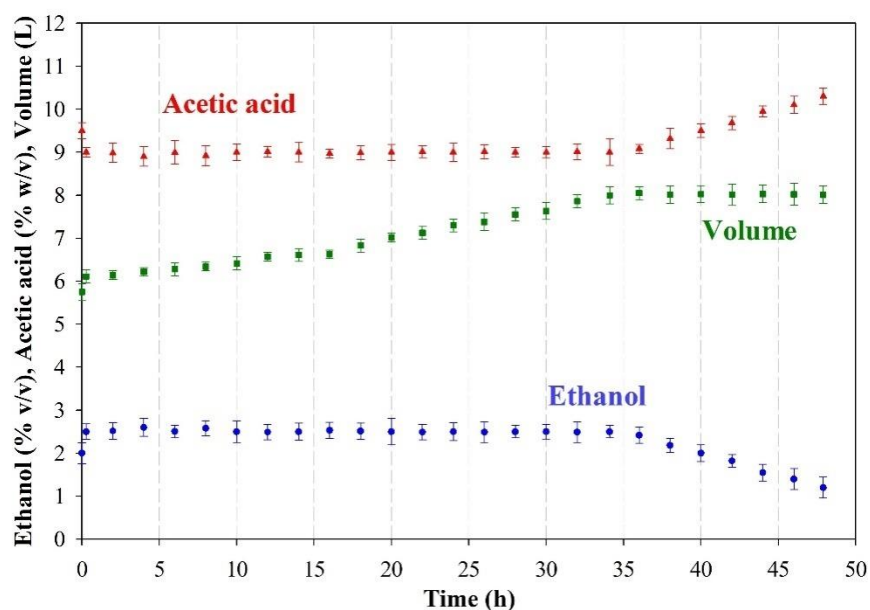
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 5.75 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 2.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 4.0 % (v/v).
- Temperature in the first bioreactor: 32 °C.
- Ethanol concentration during loading of the second bioreactor: 2.5 % (v/v).
- Temperature in the second bioreactor: 32 °C.

Figures S2.3 and S2.4 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of eight replications with their standard deviations.



**Figure S2.3.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 2.



**Figure S2.4.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 2.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.3 shows the values for Experiment 2.

**Table S2.3.** Experimental results obtained in Experiment 2.

Variable	Value
Total cycle duration (h)	48.0 ± 0.9
Duration of the fast loading stage in the first bioreactor (h)	0.5 ± 0.1
Duration of the slow loading stage in the first bioreactor (h)	43.4 ± 0.8
Duration of the depletion stage in the first bioreactor (h)	4.1 ± 1.2
Duration of the fast loading stage in the second bioreactor (h)	0.3 ± 0.1
Duration of the slow loading stage in the second bioreactor (h)	33.8 ± 0.4
Duration of the depletion stage in the second bioreactor (h)	13.8 ± 0.7
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	0.010 ± 0.001
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	0.905 ± 0.024
Time fraction of the depletion stage in the first bioreactor over the cycle duration	0.085 ± 0.025
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	0.006 ± 0.001
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	0.706 ± 0.016
Time fraction of the depletion stage in the second bioreactor over the cycle duration	0.288 ± 0.016
Mean volume in the fast loading stage in the first bioreactor (L)	2.55 ± 0.05
Mean volume in the slow loading stage in the first bioreactor (L)	5.43 ± 0.05
Mean volume in the depletion stage in the first bioreactor (L)	8.01 ± 0.05
Mean volume in the first bioreactor (L)	5.61 ± 0.24
Mean volume in the fast loading stage in the second bioreactor (L)	5.93 ± 0.05
Mean volume in the slow loading stage in the second bioreactor (L)	7.06 ± 0.05
Mean volume in the depletion stage in the second bioreactor (L)	8.00 ± 0.05
Mean volume in the second bioreactor (L)	7.32 ± 0.17
Mean overall volume during a cycle in the two bioreactors as a whole (L)	12.94 ± 0.29
Volume of fermentation medium unloaded from the second reactor (L)	8.00 ± 0.05
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	1.2 ± 0.2
Mean ethanol concentration in the first bioreactor (% v/v)	3.8 ± 0.1
Mean ethanol concentration in the second bioreactor (% v/v)	2.3 ± 0.1

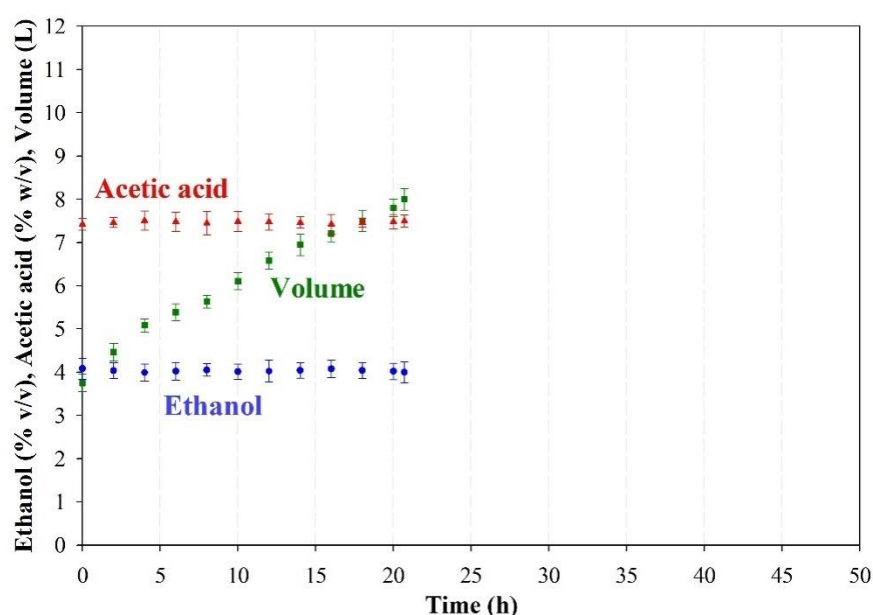
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$9.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$10.3 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$7.7 \pm 0.1$
Mean acetic acid concentration in the second bioreactor (% w/v)	$9.2 \pm 0.1$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.20 \pm 0.01$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.08 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.13 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$11.4 \pm 0.3$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$5.8 \pm 0.4$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$17.2 \pm 0.5$

### S2.3. Results of Experiment 3

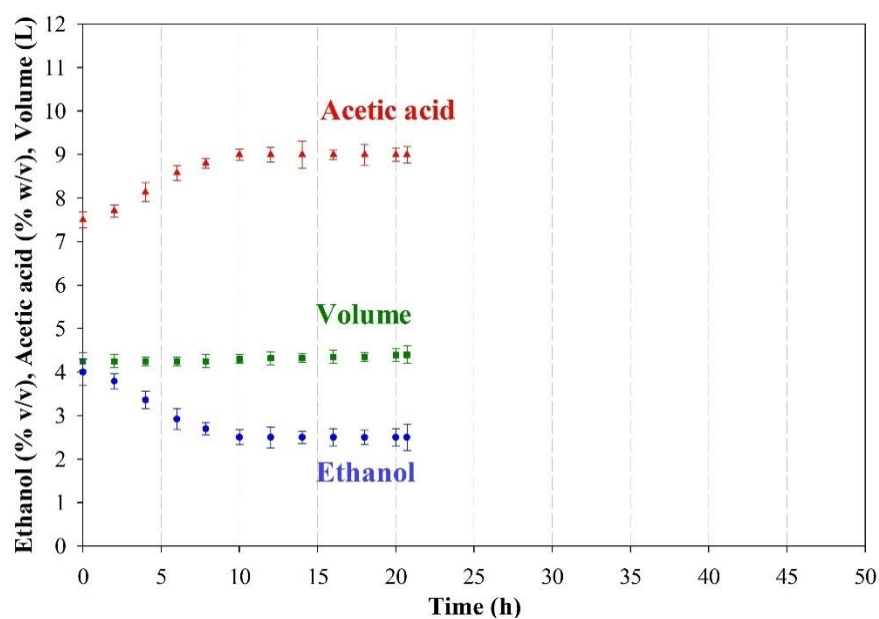
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 4.25 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 4.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 4.0 % (v/v).
- Temperature in the first bioreactor: 32 °C.
- Ethanol concentration during loading of the second bioreactor: 2.5 % (v/v).
- Temperature in the second bioreactor: 32 °C.

Figures S2.5 and S2.6 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of ten replications with their standard deviations.



**Figure S2.5.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 3.



**Figure S2.6.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 3.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.4 shows the values for Experiment 3.



**Table S2.4.** Experimental results obtained in Experiment 3.

Variable	Value
Total cycle duration (h)	$20.8 \pm 0.5$
Duration of the slow loading stage in the first bioreactor (h)	$20.8 \pm 0.5$
Duration of the previous depletion stage in the second bioreactor (h)	$7.9 \pm 0.2$
Duration of the slow loading stage in the second bioreactor (h)	$12.9 \pm 0.4$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$1.0 \pm 0.034$
Time fraction of the previous depletion stage in the second bioreactor over the cycle duration	$0.379 \pm 0.012$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.621 \pm 0.025$
Mean volume in the slow loading stage in the first bioreactor (L)	$5.89 \pm 0.05$
Mean volume in the first bioreactor (L)	$5.89 \pm 0.34$
Mean volume in the previous depletion stage in the second bioreactor (L)	$4.25 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$4.40 \pm 0.05$
Mean volume in the second bioreactor (L)	$4.30 \pm 0.12$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$10.19 \pm 0.36$
Volume of fermentation medium unloaded from the second reactor (L)	$4.40 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$2.3 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$4.0 \pm 0.1$
Mean ethanol concentration in the second bioreactor (% v/v)	$2.9 \pm 0.2$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$7.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$9.2 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$7.5 \pm 0.1$
Mean acetic acid concentration in the second bioreactor (% w/v)	$8.6 \pm 0.2$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.26 \pm 0.02$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.10 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.19 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$15.3 \pm 0.6$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$4.2 \pm 0.6$

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Total acetic acid production in the two bioreactors as a whole (g acetic acid·h <sup>-1</sup> )	19.5 ± 0.6
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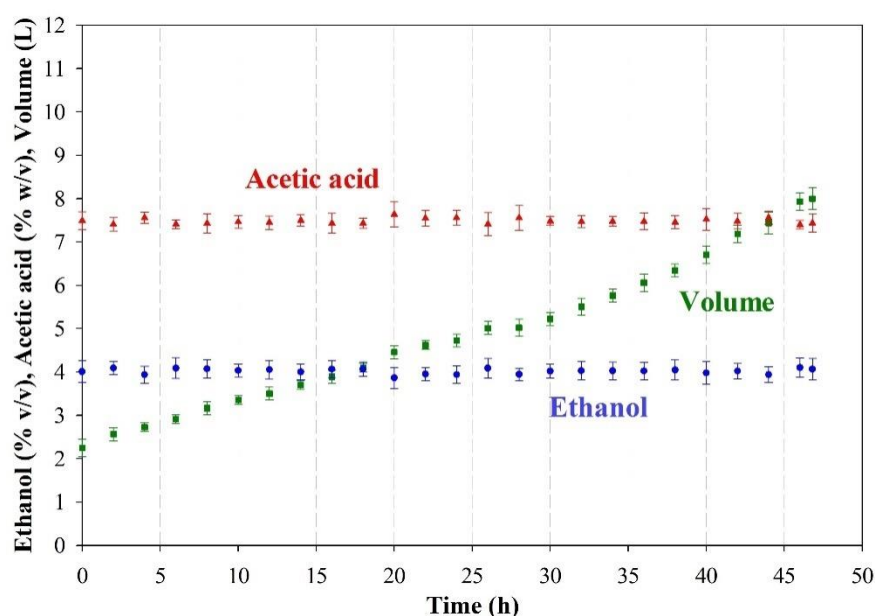
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## S2.4. Results of Experiment 4

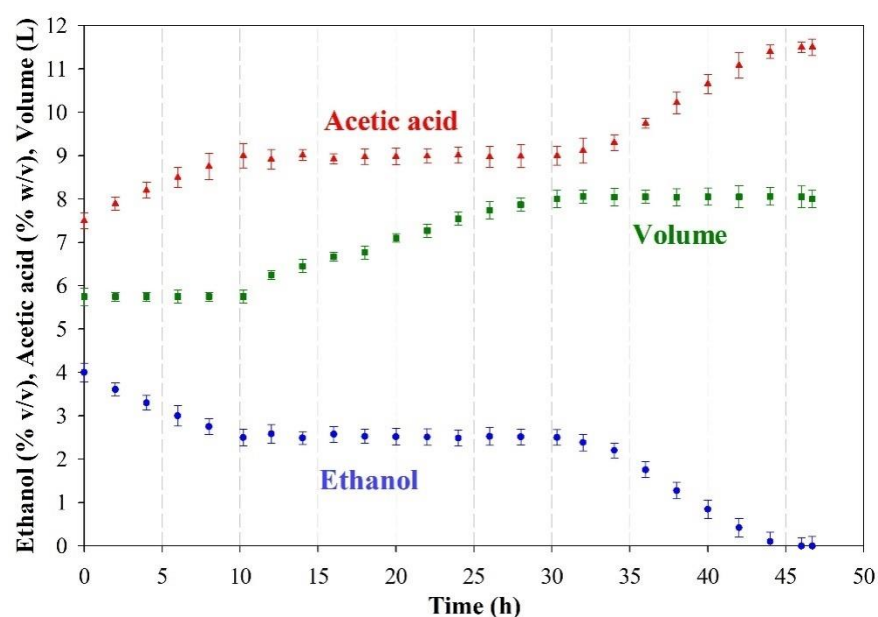
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 5.75 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 4.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 4.0 % (v/v).
- Temperature in the first bioreactor: 28 °C.
- Ethanol concentration during loading of the second bioreactor: 2.5 % (v/v).
- Temperature in the second bioreactor: 28 °C.

Figures S2.7 and S2.8 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of six replications with their standard deviations.



**Figure S2.7.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 4.



**Figure S2.8.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 4.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.5 shows the values for Experiment 4.

**Table S2.5.** Experimental results obtained in Experiment 4.

Variable	Value
Total cycle duration (h)	46.8 ± 1.2
Duration of the slow loading stage in the first bioreactor (h)	46.8 ± 1.2
Duration of the previous depletion stage in the second bioreactor (h)	10.2 ± 0.1
Duration of the slow loading stage in the second bioreactor (h)	20.1 ± 0.4
Duration of the depletion stage in the second bioreactor (h)	14.8 ± 0.8
Duration of the starving stage in the second bioreactor (h)	1.6 ± 1.1
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	1.000 ± 0.036
Time fraction of the previous depletion stage in the second bioreactor over the cycle duration	0.219 ± 0.004
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	0.431 ± 0.012
Time fraction of the depletion stage in the second bioreactor over the cycle duration	0.316 ± 0.018
Time fraction of the starving stage in the second bioreactor over the cycle duration	0.034 ± 0.023
Mean volume in the slow loading stage in the first bioreactor (L)	5.13 ± 0.05
Mean volume in the first bioreactor (L)	5.13 ± 0.19
Mean volume in the previous depletion stage in the second bioreactor (L)	5.75 ± 0.05
Mean volume in the slow loading stage in the second bioreactor (L)	6.88 ± 0.05
Mean volume in the depletion stage in the second bioreactor (L)	8.00 ± 0.05
Mean volume in the starving stage in the second bioreactor (L)	8.00 ± 0.05
Mean volume in the second bioreactor (L)	7.02 ± 0.25
Mean overall volume during a cycle in the two bioreactors as a whole (L)	12.15 ± 0.31
Volume of fermentation medium unloaded from the second reactor (L)	8.00 ± 0.05
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	0.0 ± 0.2
Mean ethanol concentration in the first bioreactor (% v/v)	4.0 ± 0.1
Mean ethanol concentration in the second bioreactor (% v/v)	2.2 ± 0.2
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	7.5 ± 0.2
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	11.5 ± 0.2
Mean acetic acid concentration in the first bioreactor (% w/v)	7.5 ± 0.1

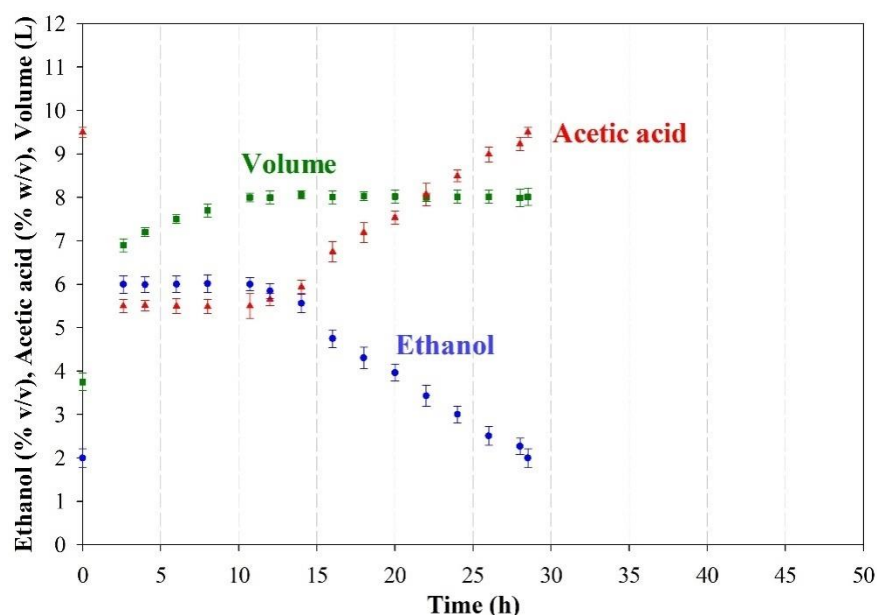
Mean acetic acid concentration in the second bioreactor (% w/v)	$9.3 \pm 0.2$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.18 \pm 0.01$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.15 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.16 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$9.2 \pm 0.3$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$10.5 \pm 0.5$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$19.7 \pm 0.6$

## S2.5. Results of Experiment 5

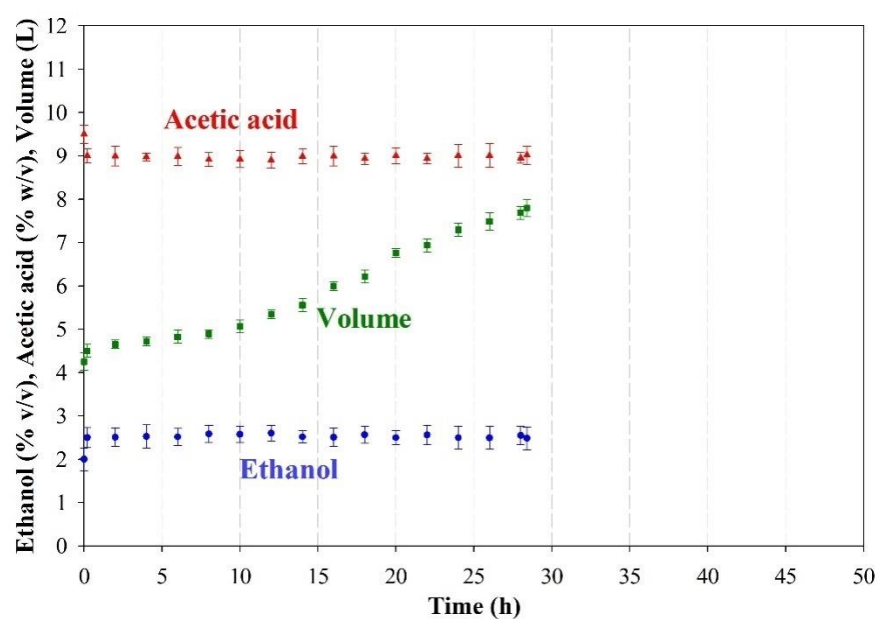
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 4.25 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 2.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 6.0 % (v/v).
- Temperature in the first bioreactor: 32 °C.
- Ethanol concentration during loading of the second bioreactor: 2.5 % (v/v).
- Temperature in the second bioreactor: 28 °C.

Figures S2.9 and S2.10 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of seven replications with their standard deviations.



**Figure S2.9.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 5.



**Figure S2.10.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 5.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.6 shows the values for Experiment 5.

**Table S2.6.** Experimental results obtained in Experiment 5.

Variable	Value
Total cycle duration (h)	$28.5 \pm 0.7$
Duration of the slow loading stage in the first bioreactor (h)	$2.6 \pm 0.1$
Duration of the slow loading stage in the first bioreactor (h)	$8.1 \pm 0.3$
Duration of the depletion stage in the first bioreactor (h)	$17.8 \pm 0.8$
Duration of the fast loading stage in the second bioreactor (h)	$0.2 \pm 0.1$
Duration of the slow loading stage in the second bioreactor (h)	$28.2 \pm 0.7$
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	$0.091 \pm 0.004$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$0.284 \pm 0.013$
Time fraction of the depletion stage in the first bioreactor over the cycle duration	$0.625 \pm 0.031$
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	$0.007 \pm 0.004$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.993 \pm 0.035$
Mean volume in the fast loading stage in the first bioreactor (L)	$5.33 \pm 0.05$
Mean volume in the slow loading stage in the first bioreactor (L)	$7.45 \pm 0.05$
Mean volume in the depletion stage in the first bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the first bioreactor (L)	$7.6 \pm 0.27$
Mean volume in the fast loading stage in the second bioreactor (L)	$4.38 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$6.15 \pm 0.10$
Mean volume in the second bioreactor (L)	$6.14 \pm 0.24$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$13.74 \pm 0.36$
Volume of fermentation medium unloaded from the second reactor (L)	$7.8 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$2.5 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$4.4 \pm 0.4$
Mean ethanol concentration in the second bioreactor (% v/v)	$2.5 \pm 0.1$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$9.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$9.0 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$7.1 \pm 0.4$

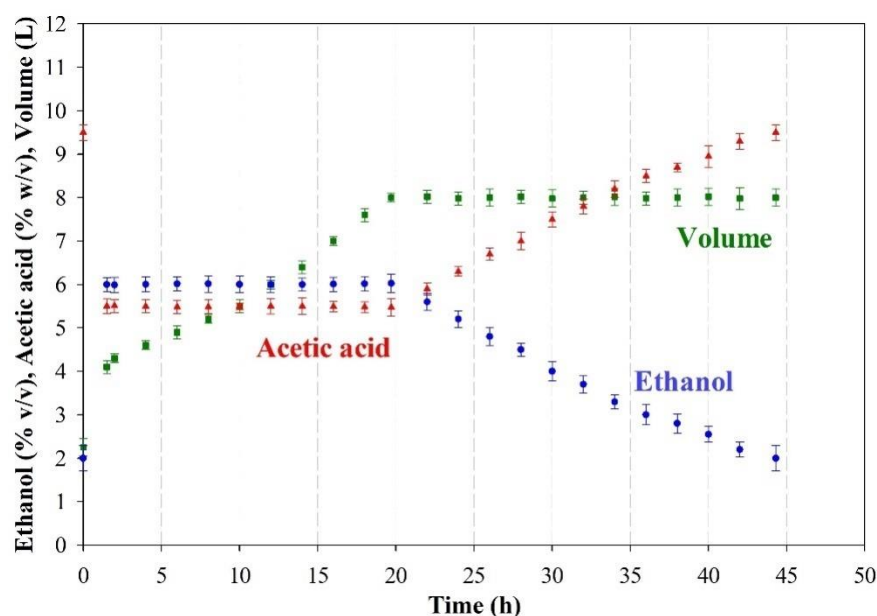
Mean acetic acid concentration in the second bioreactor (% w/v)	$9.0 \pm 0.1$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.19 \pm 0.01$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.17 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.18 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$14.2 \pm 0.5$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$10.5 \pm 0.7$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$24.6 \pm 0.8$

## S2.6. Results of Experiment 6

The operating conditions used in this experiment were as follows:

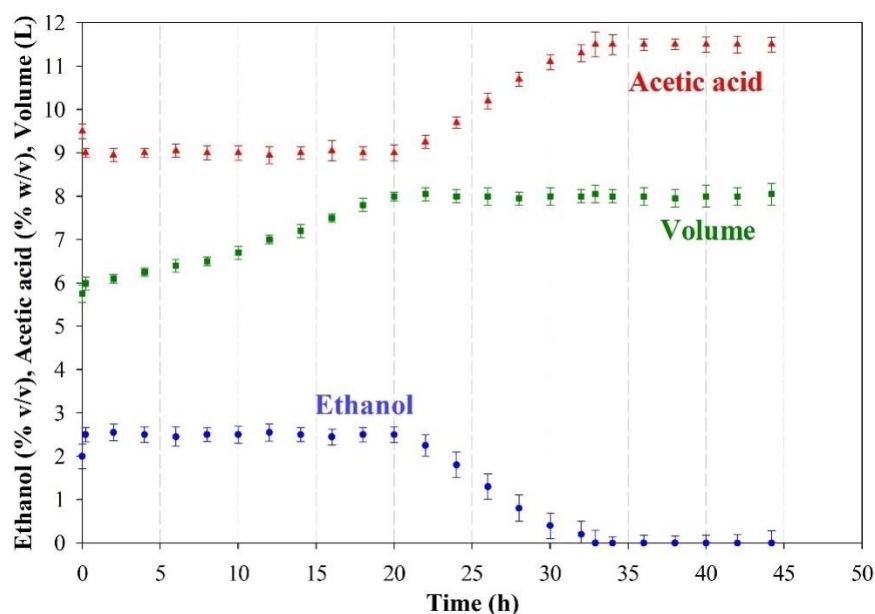
- Volume unloaded from the first bioreactor: 5.75 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 2.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 6.0 % (v/v).
- Temperature in the first bioreactor: 28 °C.
- Ethanol concentration during loading of the second bioreactor: 2.5 % (v/v).
- Temperature in the second bioreactor: 32 °C.

Figures S2.11 and S2.12 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of seven replications with their standard deviations.



**Figure S2.11.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 6.





**Figure S2.12.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 6.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.7 shows the values for Experiment 6.

**Table S2.7.** Experimental results obtained in Experiment 6.

Variable	Value
Total cycle duration (h)	$44.3 \pm 0.6$
Duration of the fast loading stage in the first bioreactor (h)	$1.5 \pm 0.1$
Duration of the slow loading stage in the first bioreactor (h)	$18.2 \pm 0.4$
Duration of the depletion stage in the first bioreactor (h)	$24.6 \pm 0.7$
Duration of the fast loading stage in the second bioreactor (h)	$0.2 \pm 0.1$
Duration of the slow loading stage in the second bioreactor (h)	$19.8 \pm 0.4$
Duration of the depletion stage in the second bioreactor (h)	$12.9 \pm 0.7$
Duration of the starving stage in the second bioreactor (h)	$11.3 \pm 0.8$
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	$0.034 \pm 0.002$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$0.411 \pm 0.011$
Time fraction of the depletion stage in the first bioreactor over the cycle duration	$0.555 \pm 0.018$
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	$0.005 \pm 0.002$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.448 \pm 0.011$
Time fraction of the depletion stage in the second bioreactor over the cycle duration	$0.292 \pm 0.017$

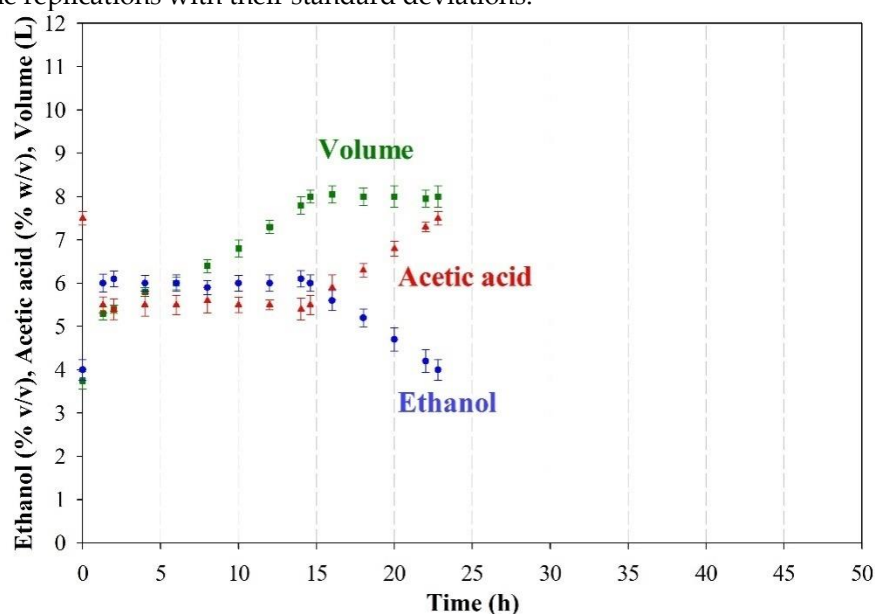
Time fraction of the starving stage in the second bioreactor over the cycle duration	$0.256 \pm 0.020$
Mean volume in the fast loading stage in the first bioreactor (L)	$3.18 \pm 0.05$
Mean volume in the slow loading stage in the first bioreactor (L)	$6.05 \pm 0.05$
Mean volume in the depletion stage in the first bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the first bioreactor (L)	$7.04 \pm 0.16$
Mean volume in the fast loading stage in the second bioreactor (L)	$5.87 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$7.00 \pm 0.05$
Mean volume in the depletion stage in the second bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the starving stage in the second bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the second bioreactor (L)	$7.54 \pm 0.22$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$14.58 \pm 0.27$
Volume of fermentation medium unloaded from the second reactor (L)	$8.00 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$0.0 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$4.7 \pm 0.3$
Mean ethanol concentration in the second bioreactor (% v/v)	$1.9 \pm 0.2$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$9.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$11.5 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$6.8 \pm 0.3$
Mean acetic acid concentration in the second bioreactor (% w/v)	$9.6 \pm 0.2$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.18 \pm 0.01$
Mean rate of acetic acid formation in the second bioreactor (% w/v · h <sup>-1</sup> )	$0.11 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.14 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$12.3 \pm 0.3$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$8.5 \pm 0.5$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$20.8 \pm 0.5$

## S2.7. Results of Experiment 7

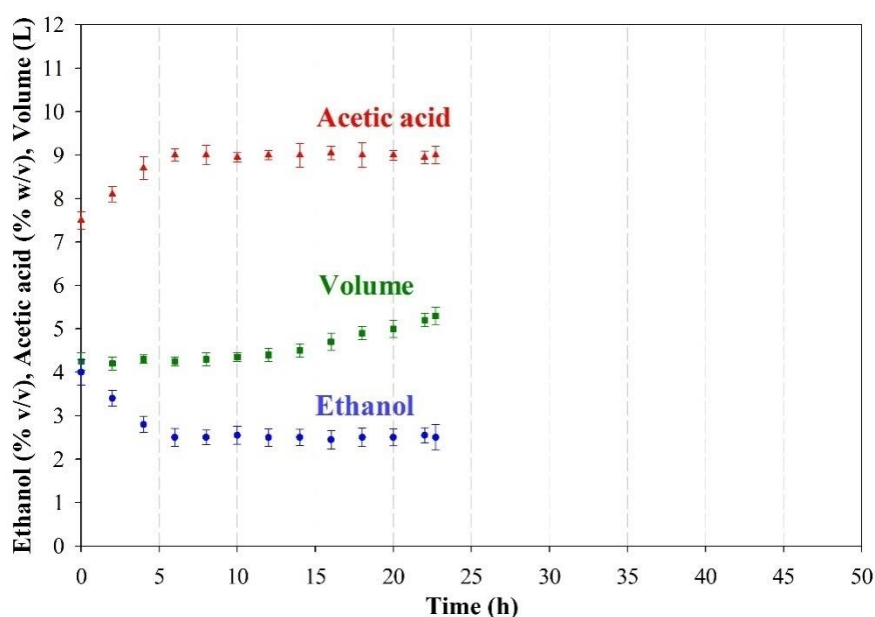
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 4.25 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 4.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 6.0 % (v/v).
- Temperature in the first bioreactor: 28 °C.
- Ethanol concentration during loading of the second bioreactor: 2.5 % (v/v).
- Temperature in the second bioreactor: 32 °C.

Figures S2.13 and S2.14 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of nine replications with their standard deviations.



**Figure S2.13.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 7.



**Figure S2.14.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 7.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.8 shows the values for Experiment 7.

**Table S2.8.** Experimental results obtained in Experiment 7.

Variable	Value
Total cycle duration (h)	$22.8 \pm 0.7$
Duration of the fast loading stage in the first bioreactor (h)	$1.3 \pm 0.1$
Duration of the slow loading stage in the first bioreactor (h)	$13.3 \pm 0.3$
Duration of the depletion stage in the first bioreactor (h)	$8.2 \pm 0.8$
Duration of the previous depletion stage in the second bioreactor (h)	$6.0 \pm 0.4$
Duration of the slow loading stage in the second bioreactor (h)	$16.7 \pm 0.7$
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	$0.057 \pm 0.005$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$0.583 \pm 0.023$
Time fraction of the depletion stage in the first bioreactor over the cycle duration	$0.360 \pm 0.035$
Time fraction of the depletion stage in the second bioreactor over the cycle duration	$0.264 \pm 0.019$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.736 \pm 0.037$
Mean volume in the fast loading stage in the first bioreactor (L)	$4.53 \pm 0.05$
Mean volume in the slow loading stage in the first bioreactor (L)	$6.65 \pm 0.05$
Mean volume in the depletion stage in the first bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the first bioreactor (L)	$7.01 \pm 0.32$
Mean volume in the previous depletion stage in the second bioreactor (L)	$4.25 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$4.78 \pm 0.05$
Mean volume in the second bioreactor (L)	$4.64 \pm 0.20$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$11.65 \pm 0.37$
Volume of fermentation medium unloaded from the second reactor (L)	$5.3 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$2.5 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$5.5 \pm 0.2$
Mean ethanol concentration in the second bioreactor (% v/v)	$2.7 \pm 0.1$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$7.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$9.0 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$6.0 \pm 0.2$

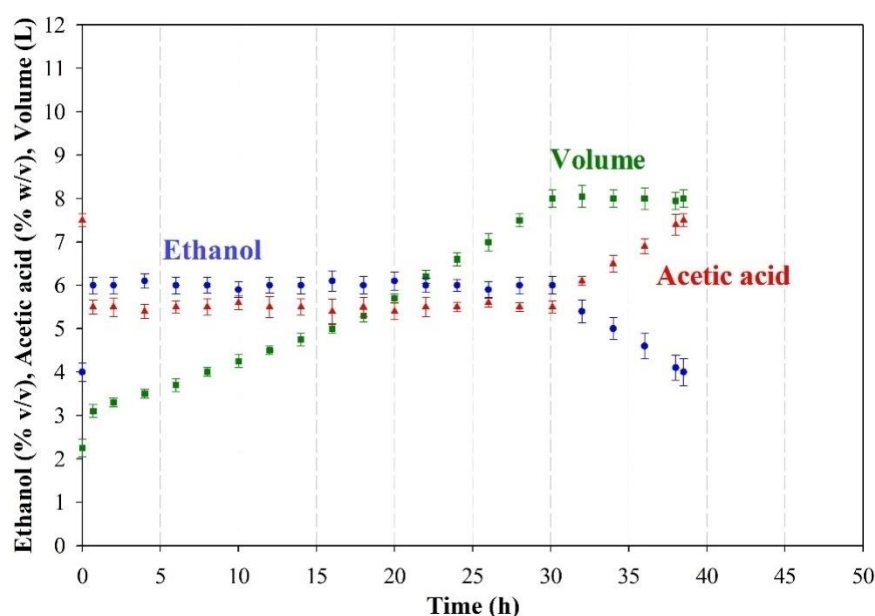
Mean acetic acid concentration in the second bioreactor (% w/v)	$8.8 \pm 0.1$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.20 \pm 0.01$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.15 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.18 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$14.0 \pm 0.6$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$7.0 \pm 0.6$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$20.9 \pm 0.8$

## S2.8. Results of Experiment 8

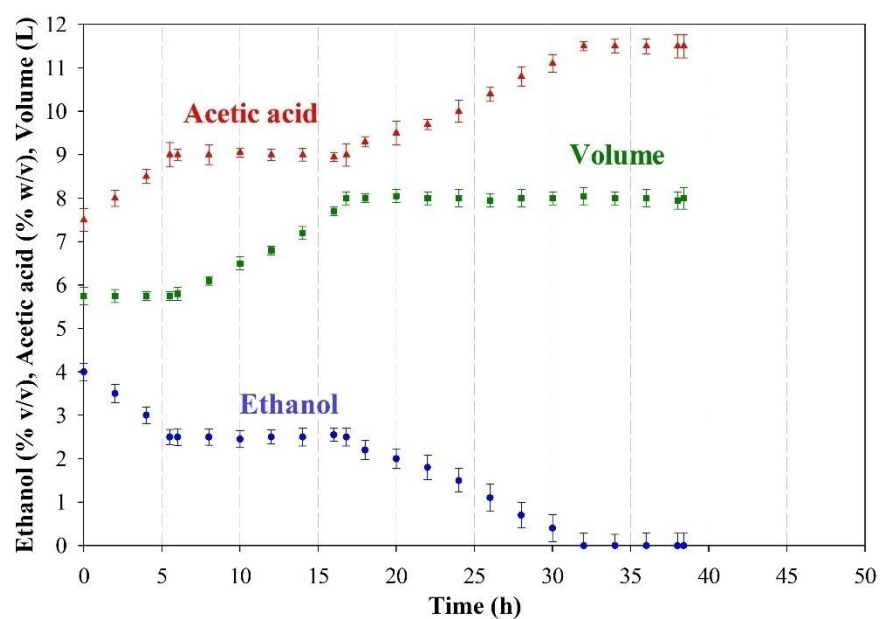
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 5.75 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 4.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 6.0 % (v/v).
- Temperature in the first bioreactor: 32 °C.
- Ethanol concentration during loading of the second bioreactor: 2.5 % (v/v).
- Temperature in the second bioreactor: 28 °C.

Figures S2.15 and S2.16 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of five replications with their standard deviations.



**Figure S2.15.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 8.



**Figure S2.16.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 8.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.9 shows the values for Experiment 8.

**Table S2.9.** Experimental results obtained in Experiment 8.

Variable	Value
Total cycle duration (h)	$38.5 \pm 0.5$
Duration of the fast loading stage in the first bioreactor (h)	$0.7 \pm 0.1$
Duration of the slow loading stage in the first bioreactor (h)	$29.4 \pm 0.3$
Duration of the depletion stage in the first bioreactor (h)	$8.4 \pm 0.6$
Duration of the previous depletion stage in the second bioreactor (h)	$5.5 \pm 0.2$
Duration of the slow loading stage in the second bioreactor (h)	$11.3 \pm 0.4$
Duration of the depletion stage in the second bioreactor (h)	$15.2 \pm 0.8$
Duration of the starving stage in the second bioreactor (h)	$6.4 \pm 1.0$
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	$0.018 \pm 0.003$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$0.764 \pm 0.013$
Time fraction of the depletion stage in the first bioreactor over the cycle duration	$0.218 \pm 0.015$
Time fraction of the previous depletion stage in the second bioreactor over the cycle duration	$0.143 \pm 0.006$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.294 \pm 0.013$
Time fraction of the depletion stage in the second bioreactor over the cycle duration	$0.396 \pm 0.022$
Time fraction of the starving stage in the second bioreactor over the cycle duration	$0.167 \pm 0.026$
Mean volume in the fast loading stage in the first bioreactor (L)	$2.68 \pm 0.05$
Mean volume in the slow loading stage in the first bioreactor (L)	$5.55 \pm 0.05$
Mean volume in the depletion stage in the first bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the first bioreactor (L)	$6.03 \pm 0.14$
Mean volume in the previous depletion stage in the second bioreactor (L)	$5.75 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$6.88 \pm 0.05$
Mean volume in the depletion stage in the second bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the starving stage in the second bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the second bioreactor (L)	$7.35 \pm 0.29$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$13.38 \pm 0.32$
Volume of fermentation medium unloaded from the second reactor (L)	$8.00 \pm 0.05$

Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$0.0 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$5.6 \pm 0.2$
Mean ethanol concentration in the second bioreactor (% v/v)	$2.1 \pm 0.2$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$7.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$11.5 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$5.9 \pm 0.2$
Mean acetic acid concentration in the second bioreactor (% w/v)	$9.4 \pm 0.2$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.19 \pm 0.01$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.17 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.18 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$11.2 \pm 0.3$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$12.7 \pm 0.6$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$23.9 \pm 0.5$

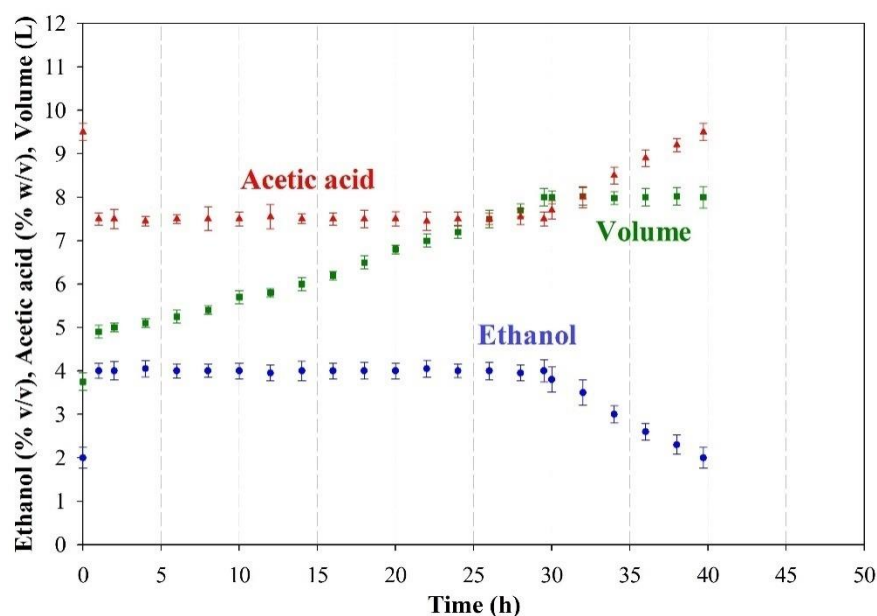
## S2.9. Results of Experiment 9

The operating conditions used in this experiment were as follows:

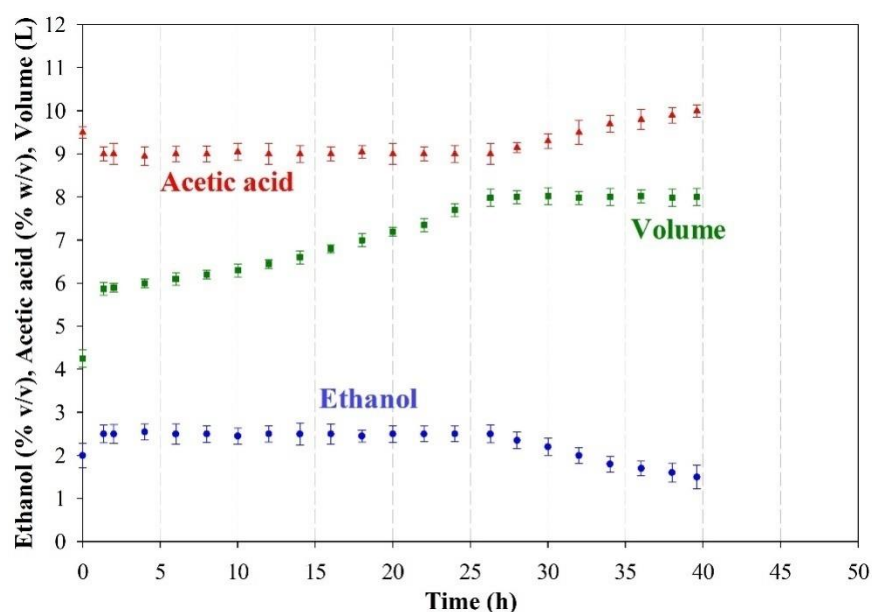
- Volume unloaded from the first bioreactor: 4.25 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 2.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 4.0 % (v/v).
- Temperature in the first bioreactor: 28 °C.
- Ethanol concentration during loading of the second bioreactor: 4.5 % (v/v).
- Temperature in the second bioreactor: 32 °C.

Figures S2.17 and S2.18 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of thirteen replications with their standard deviations.





**Figure S2.17.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 9.



**Figure S2.18.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 9.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.10 shows the values for Experiment 9.

**Table S2.10.** Experimental results obtained in Experiment 9.

Variable	Value
Total cycle duration (h)	$39.7 \pm 0.9$
Duration of the fast loading stage in the first bioreactor (h)	$1.0 \pm 0.1$
Duration of the slow loading stage in the first bioreactor (h)	$28.5 \pm 0.7$
Duration of the depletion stage in the first bioreactor (h)	$10.2 \pm 1.1$

Duration of the fast loading stage in the second bioreactor (h)	$1.4 \pm 0.1$
Duration of the slow loading stage in the second bioreactor (h)	$24.9 \pm 0.3$
Duration of the depletion stage in the second bioreactor (h)	$13.3 \pm 0.7$
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	$0.025 \pm 0.003$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$0.718 \pm 0.024$
Time fraction of the depletion stage in the first bioreactor over the cycle duration	$0.257 \pm 0.029$
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	$0.034 \pm 0.003$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.630 \pm 0.013$
Time fraction of the depletion stage in the second bioreactor over the cycle duration	$0.336 \pm 0.018$
Mean volume in the fast loading stage in the first bioreactor (L)	$4.33 \pm 0.05$
Mean volume in the slow loading stage in the first bioreactor (L)	$6.46 \pm 0.05$
Mean volume in the depletion stage in the first bioreactor (L)	$8.02 \pm 0.05$
Mean volume in the first bioreactor (L)	$6.81 \pm 0.28$
Mean volume in the fast loading stage in the second bioreactor (L)	$5.06 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$6.93 \pm 0.05$
Mean volume in the depletion stage in the second bioreactor (L)	$7.98 \pm 0.05$
Mean volume in the second bioreactor (L)	$7.22 \pm 0.17$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$14.02 \pm 0.33$
Volume of fermentation medium unloaded from the second reactor (L)	$7.98 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$1.5 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$3.6 \pm 0.1$
Mean ethanol concentration in the second bioreactor (% v/v)	$2.3 \pm 0.1$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$9.6 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$10.0 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$7.9 \pm 0.1$
Mean acetic acid concentration in the second bioreactor (% w/v)	$9.2 \pm 0.1$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.15 \pm 0.01$

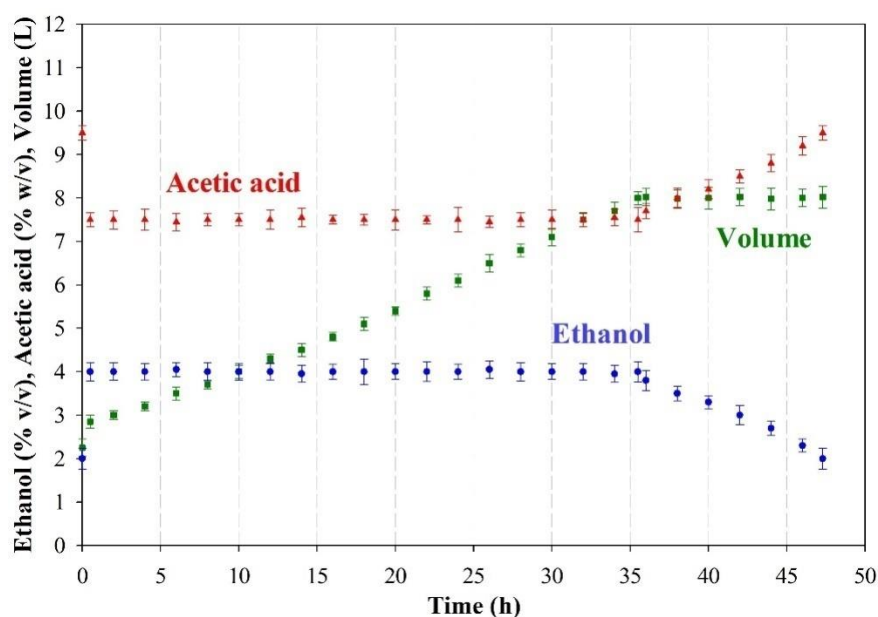
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	0.14 ± 0.01
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	0.14 ± 0.01
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	10.3 ± 0.3
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	9.9 ± 0.5
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	20.1 ± 0.6

## S2.10. Results of Experiment 10

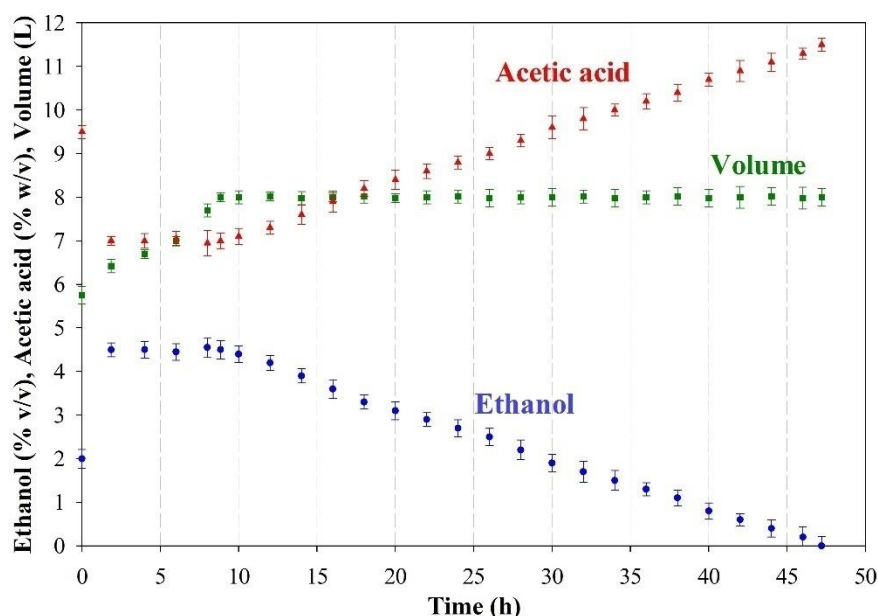
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 5.75 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 2.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 4.0 % (v/v).
- Temperature in the first bioreactor: 32 °C.
- Ethanol concentration during loading of the second bioreactor: 4.5 % (v/v).
- Temperature in the second bioreactor: 28 °C.

Figures S2.19 and S2.20 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of five replications with their standard deviations.



**Figure S2.19.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 10.



**Figure S2.20.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 10.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.11 shows the values for Experiment 10.

**Table S2.11.** Experimental results obtained in Experiment 10

Variable	Value
Total cycle duration (h)	47.3 ± 0.6
Duration of the fast loading stage in the first bioreactor (h)	0.5 ± 0.1
Duration of the slow loading stage in the first bioreactor (h)	35.0 ± 0.4
Duration of the depletion stage in the first bioreactor (h)	11.8 ± 0.7
Duration of the fast loading stage in the second bioreactor (h)	1.9 ± 0.1
Duration of the slow loading stage in the second bioreactor (h)	7.0 ± 0.4
Duration of the depletion stage in the second bioreactor (h)	38.4 ± 0.8
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	0.011 ± 0.002
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	0.740 ± 0.013
Time fraction of the depletion stage in the first bioreactor over the cycle duration	0.250 ± 0.016
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	0.039 ± 0.001
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	0.148 ± 0.009
Time fraction of the depletion stage in the second bioreactor over the cycle duration	0.813 ± 0.021
Mean volume in the fast loading stage in the first bioreactor (L)	2.55 ± 0.05

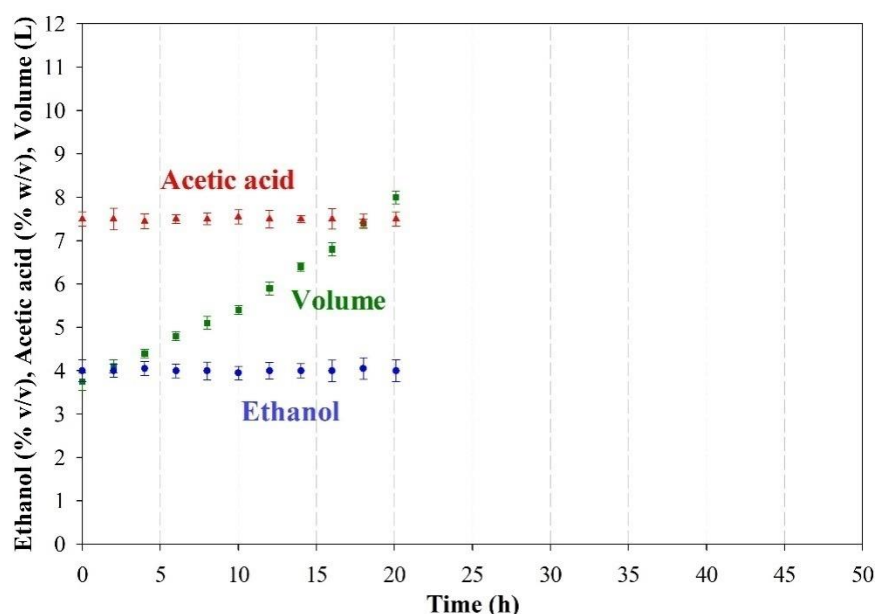
Mean volume in the slow loading stage in the first bioreactor (L)	$5.43 \pm 0.05$
Mean volume in the depletion stage in the first bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the first bioreactor (L)	$6.04 \pm 0.14$
Mean volume in the fast loading stage in the second bioreactor (L)	$6.68 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$7.80 \pm 0.05$
Mean volume in the depletion stage in the second bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the second bioreactor (L)	$7.92 \pm 0.18$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$13.96 \pm 0.23$
Volume of fermentation medium unloaded from the second reactor (L)	$8.00 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$0.0 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$3.7 \pm 0.1$
Mean ethanol concentration in the second bioreactor (% v/v)	$2.6 \pm 0.3$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$9.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$11.6 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$7.8 \pm 0.1$
Mean acetic acid concentration in the second bioreactor (% w/v)	$8.9 \pm 0.3$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.19 \pm 0.01$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.10 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.14 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$11.5 \pm 0.3$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$8.1 \pm 0.4$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$19.6 \pm 0.4$

## S2.11. Results of Experiment 11

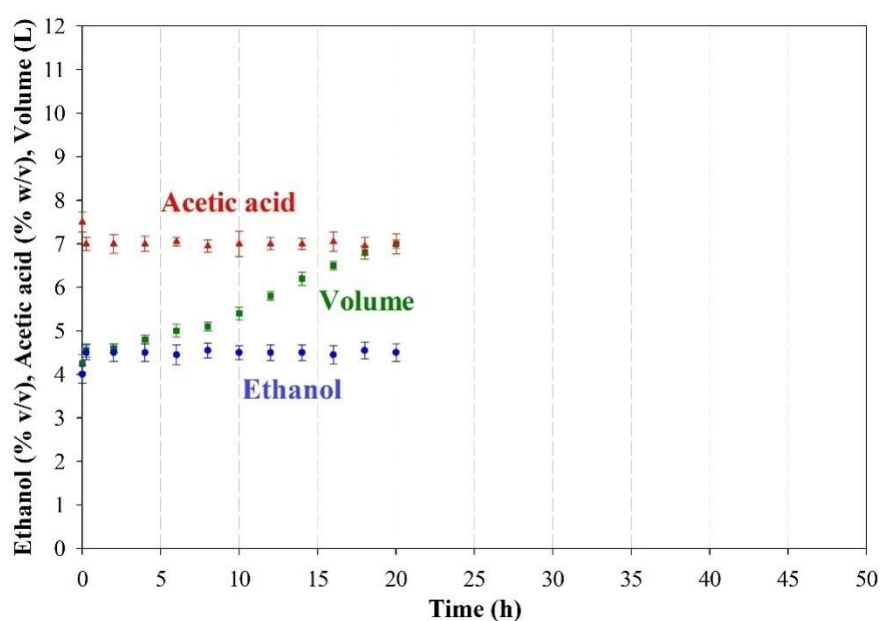
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 4.25 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 4.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 4.0 % (v/v).
- Temperature in the first bioreactor: 32 °C.
- Ethanol concentration during loading of the second bioreactor: 4.5 % (v/v).
- Temperature in the second bioreactor: 28 °C.

Figures S2.21 and S2.22 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of thirteen replications with their standard deviations.



**Figure S2.21.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 11.



**Figure S2.22.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 11.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.12 shows the values for Experiment 11.

**Table S2.12.** Experimental results obtained in Experiment 11

Variable	Value
Total cycle duration (h)	20.1 ± 0.6

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Duration of the slow loading stage in the first bioreactor (h)	$20.1 \pm 0.6$
Duration of the fast loading stage in the second bioreactor (h)	$0.3 \pm 0.1$
Duration of the slow loading stage in the second bioreactor (h)	$19.8 \pm 0.6$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$1.0 \pm 0.042$
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	$0.013 \pm 0.005$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.998 \pm 0.042$
Mean volume in the slow loading stage in the first bioreactor (L)	$5.89 \pm 0.05$
Mean volume in the first bioreactor (L)	$5.89 \pm 0.42$
Mean volume in the fast loading stage in the second bioreactor (L)	$4.4 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$5.78 \pm 0.05$
Mean volume in the second bioreactor (L)	$5.76 \pm 0.25$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$11.65 \pm 0.49$
Volume of fermentation medium unloaded from the second reactor (L)	$7.00 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$4.5 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$4.0 \pm 0.1$
Mean ethanol concentration in the second bioreactor (% v/v)	$4.5 \pm 0.1$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$7.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$7.0 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$7.5 \pm 0.1$
Mean acetic acid concentration in the second bioreactor (% w/v)	$7.0 \pm 0.1$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.27 \pm 0.02$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.15 \pm 0.02$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.21 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$15.9 \pm 0.6$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$8.6 \pm 0.9$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$24.4 \pm 1.0$

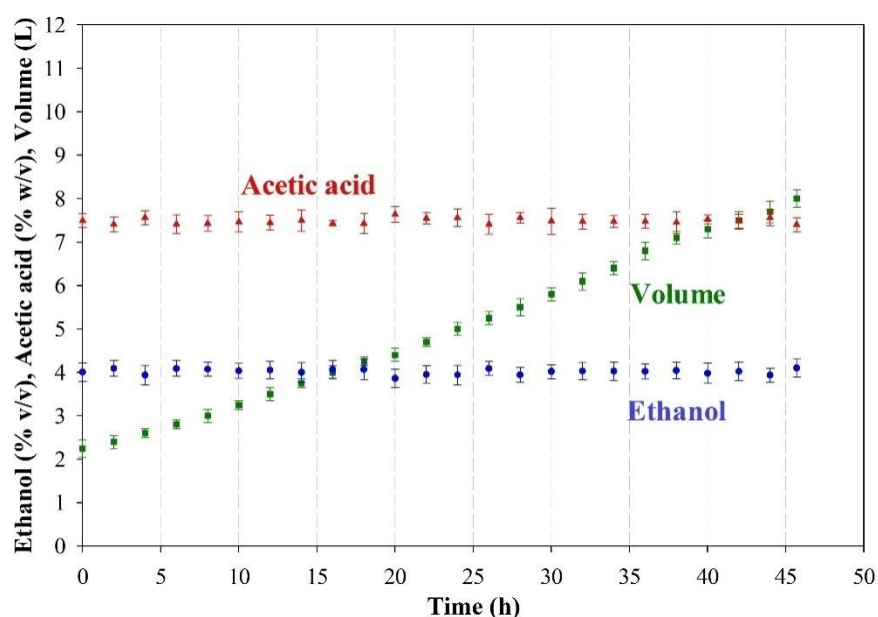
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### S2.12. Results of Experiment 12

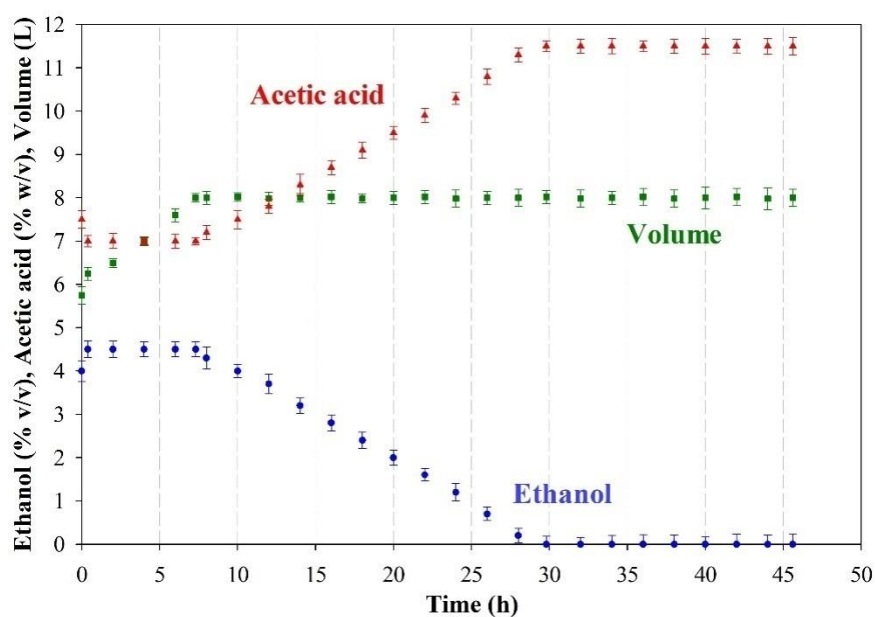
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 5.75 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 4.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 4.0 % (v/v).
- Temperature in the first bioreactor: 28 °C.
- Ethanol concentration during loading of the second bioreactor: 4.5 % (v/v).
- Temperature in the second bioreactor: 32 °C.

Figures S2.23 and S2.24 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of ten replications with their standard deviations.



**Figure S2.23.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 12.



**Figure S2.24.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 12.



The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.13 shows the values for Experiment 12.

**Table S2.13.** Experimental results obtained in Experiment 12

Variable	Value
Total cycle duration (h)	45.7 ± 1.2
Duration of the slow loading stage in the first bioreactor (h)	45.7 ± 1.2
Duration of the fast loading stage in the second bioreactor (h)	0.4 ± 0.1
Duration of the slow loading stage in the second bioreactor (h)	6.9 ± 0.6
Duration of the depletion stage in the second bioreactor (h)	22.5 ± 1.0
Duration of starving in stage the second bioreactor (h)	15.8 ± 1.3
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	1.000 ± 0.037
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	0.009 ± 0.002
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	0.151 ± 0.014
Time fraction of the depletion stage in the second bioreactor over the cycle duration	0.493 ± 0.024
Time fraction of the starving stage in the second bioreactor over the cycle duration	0.346 ± 0.029
Mean volume in the slow loading stage in the first bioreactor (L)	5.13 ± 0.05
Mean volume in the first bioreactor (L)	5.13 ± 0.19
Mean volume in the fast loading stage in the second bioreactor (L)	6.00 ± 0.05
Mean volume in the slow loading stage in the second bioreactor (L)	7.13 ± 0.05
Mean volume in the depletion stage in the second bioreactor (L)	8.00 ± 0.05
Mean volume in the starving stage in the second bioreactor (L)	8.00 ± 0.05
Mean volume in the second bioreactor (L)	7.85 ± 0.32
Mean overall volume during a cycle in the two bioreactors as a whole (L)	12.98 ± 0.37
Volume of fermentation medium unloaded from the second reactor (L)	8.00 ± 0.05
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	0.0 ± 0.2
Mean ethanol concentration in the first bioreactor (% v/v)	4.0 ± 0.1
Mean ethanol concentration in the second bioreactor (% v/v)	2.9 ± 0.4
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	7.5 ± 0.2
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	11.5 ± 0.2
Mean acetic acid concentration in the first bioreactor (% w/v)	7.5 ± 0.1

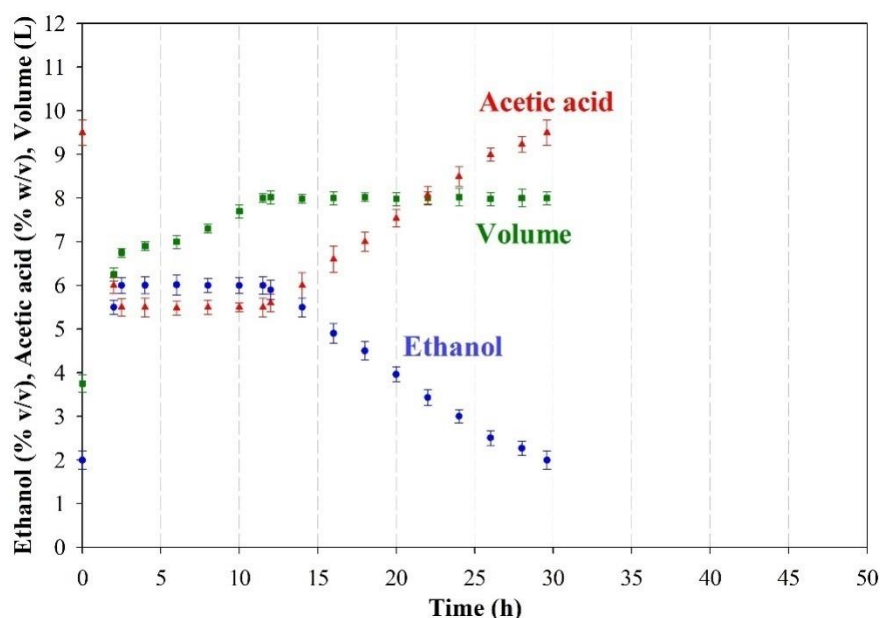
Mean acetic acid concentration in the second bioreactor (% w/v)	$8.6 \pm 0.4$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.18 \pm 0.01$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.14 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.16 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$9.4 \pm 0.4$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$10.7 \pm 0.5$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$20.1 \pm 0.6$

### S2.13. Results of Experiment 13

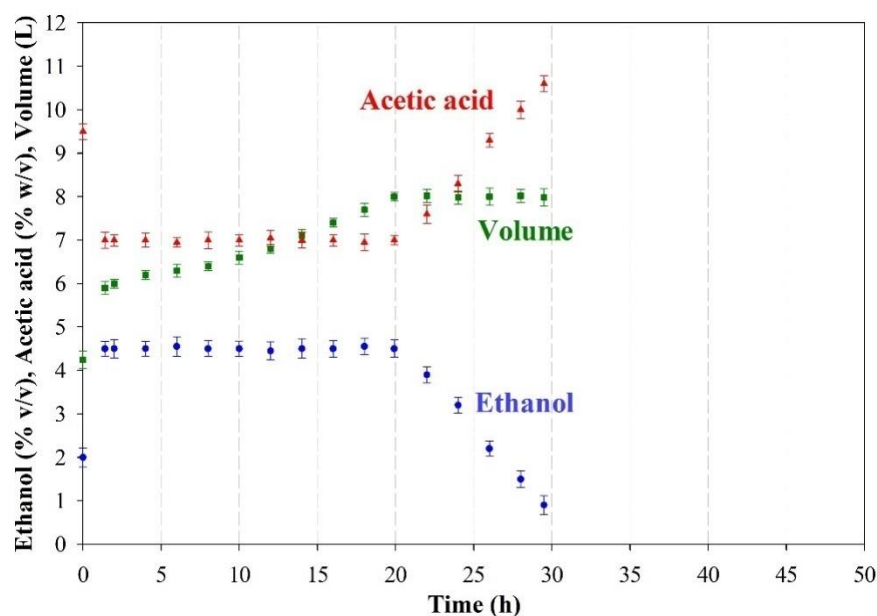
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 4.25 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 2.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 6.0 % (v/v).
- Temperature in the first bioreactor: 32 °C.
- Ethanol concentration during loading of the second bioreactor: 4.5 % (v/v).
- Temperature in the second bioreactor: 32 °C.

Figures S2.25 and S2.26 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of twelve replications with their standard deviations.



**Figure S2.25.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 13.



**Figure S2.26.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 13.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.14 shows the values for Experiment 13.

**Table S2.14.** Experimental results obtained in Experiment 13

Variable	Value
Total cycle duration (h)	$29.6 \pm 0.8$
Duration of the fast loading stage in the first bioreactor (h)	$2.5 \pm 0.1$
Duration of the slow loading stage in the first bioreactor (h)	$9.0 \pm 0.6$
Duration of the depletion stage in the first bioreactor (h)	$18.1 \pm 1.0$
Duration of the fast loading stage in the second bioreactor (h)	$1.4 \pm 0.3$
Duration of the slow loading stage in the second bioreactor (h)	$18.5 \pm 0.6$
Duration of the depletion stage in the second bioreactor (h)	$9.6 \pm 0.9$
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	$0.084 \pm 0.004$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$0.304 \pm 0.022$
Time fraction of the depletion stage in the first bioreactor over the cycle duration	$0.611 \pm 0.038$
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	$0.047 \pm 0.010$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.627 \pm 0.025$
Time fraction of the depletion stage in the second bioreactor over the cycle duration	$0.325 \pm 0.030$
Mean volume in the fast loading stage in the first bioreactor (L)	$5.25 \pm 0.05$

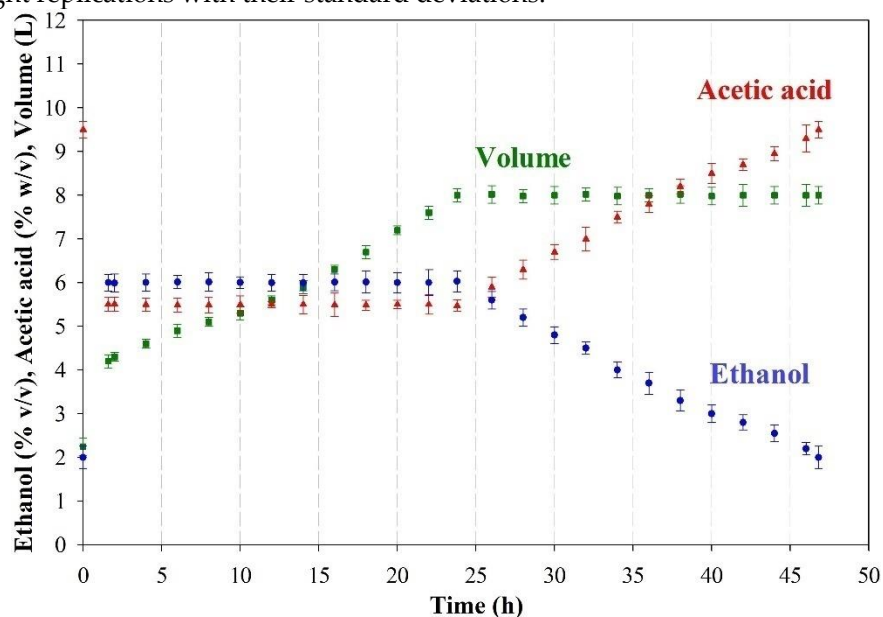
Mean volume in the slow loading stage in the first bioreactor (L)	$7.38 \pm 0.05$
Mean volume in the depletion stage in the first bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the first bioreactor (L)	$7.58 \pm 0.34$
Mean volume in the fast loading stage in the second bioreactor (L)	$5.08 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$6.95 \pm 0.05$
Mean volume in the depletion stage in the second bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the second bioreactor (L)	$7.20 \pm 0.30$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$14.78 \pm 0.46$
Volume of fermentation medium unloaded from the second reactor (L)	$8.00 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$0.9 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$4.5 \pm 0.4$
Mean ethanol concentration in the second bioreactor (% v/v)	$3.7 \pm 0.3$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$9.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$10.6 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$7.0 \pm 0.4$
Mean acetic acid concentration in the second bioreactor (% w/v)	$7.8 \pm 0.3$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.18 \pm 0.01$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.21 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.19 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$13.6 \pm 0.5$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$15.1 \pm 0.7$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$28.6 \pm 0.9$

## S2.14. Results of Experiment 14

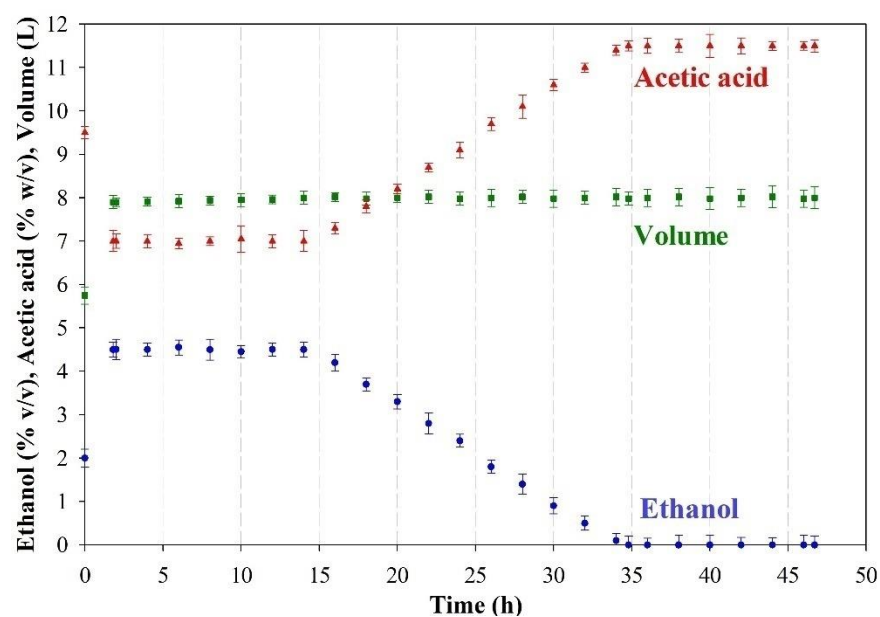
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 5.75 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 2.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 6.0 % (v/v).
- Temperature in the first bioreactor: 28 °C.
- Ethanol concentration during loading of the second bioreactor: 4.5 % (v/v).
- Temperature in the second bioreactor: 28 °C.

Figures S2.27 and S2.28 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of eight replications with their standard deviations.



**Figure S2.27.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 14.



**Figure S2.28.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 14.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.15 shows the values for Experiment 14.

**Table S2.15.** Experimental results obtained in Experiment 14

Variable	Value
Total cycle duration (h)	46.8 ± 1.1
Duration of the fast loading stage in the first bioreactor (h)	1.6 ± 0.1
Duration of the slow loading stage in the first bioreactor (h)	22.2 ± 0.9
Duration of the depletion stage in the first bioreactor (h)	23.0 ± 1.4

Duration of the fast loading stage in the second bioreactor (h)	1.8 ± 0.2
Duration of the slow loading stage in the second bioreactor (h)	12.2 ± 0.4
Duration of the depletion stage in the second bioreactor (h)	20.8 ± 0.8
Duration of starving in stage the second bioreactor (h)	11.9 ± 1.1
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	0.034 ± 0.002
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	0.474 ± 0.022
Time fraction of the depletion stage in the first bioreactor over the cycle duration	0.491 ± 0.032
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	0.039 ± 0.004
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	0.261 ± 0.009
Time fraction of the depletion stage in the second bioreactor over the cycle duration	0.445 ± 0.018
Time fraction of the starving stage in the second bioreactor over the cycle duration	0.255 ± 0.023
Mean volume in the fast loading stage in the first bioreactor (L)	3.23 ± 0.05
Mean volume in the slow loading stage in the first bioreactor (L)	6.10 ± 0.05
Mean volume in the depletion stage in the first bioreactor (L)	8.00 ± 0.05
Mean volume in the first bioreactor (L)	6.94 ± 0.29
Mean volume in the fast loading stage in the second bioreactor (L)	6.83 ± 0.05
Mean volume in the slow loading stage in the second bioreactor (L)	7.95 ± 0.05
Mean volume in the depletion stage in the second bioreactor (L)	8.00 ± 0.05
Mean volume in the starving stage in the second bioreactor (L)	8.00 ± 0.05
Mean volume in the second bioreactor (L)	7.94 ± 0.26
Mean overall volume during a cycle in the two bioreactors as a whole (L)	14.88 ± 0.39
Volume of fermentation medium unloaded from the second reactor (L)	8.00 ± 0.05
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	0.0 ± 0.2
Mean ethanol concentration in the first bioreactor (% v/v)	4.8 ± 0.3
Mean ethanol concentration in the second bioreactor (% v/v)	3.0 ± 0.4
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	9.5 ± 0.2
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	11.5 ± 0.2
Mean acetic acid concentration in the first bioreactor (% w/v)	6.7 ± 0.3
Mean acetic acid concentration in the second bioreactor (% w/v)	8.5 ± 0.4
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	0.17 ± 0.01
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	0.10 ± 0.01
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	0.13 ± 0.01
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	11.7 ± 0.4
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	8.0 ± 0.4
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	19.7 ± 0.6

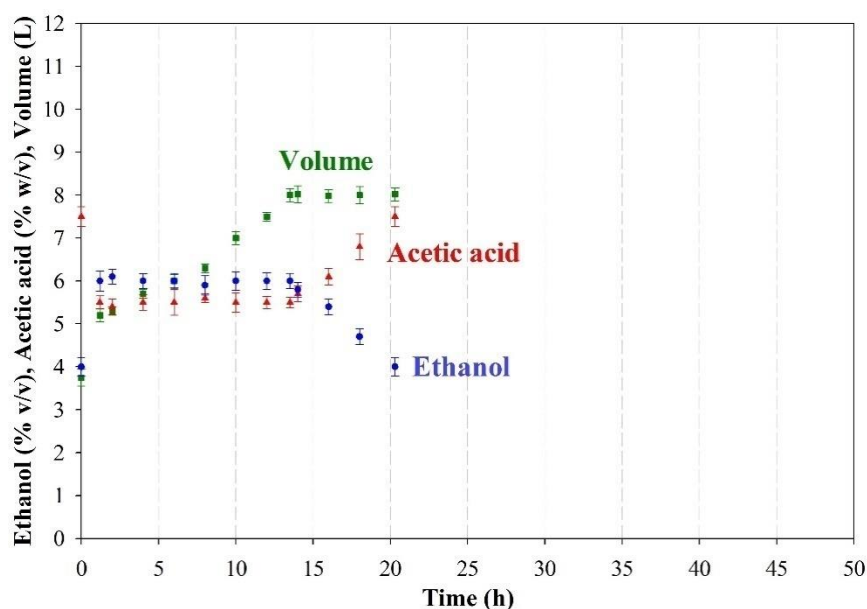
## S2.15. Results of Experiment 15

The operating conditions used in this experiment were as follows:

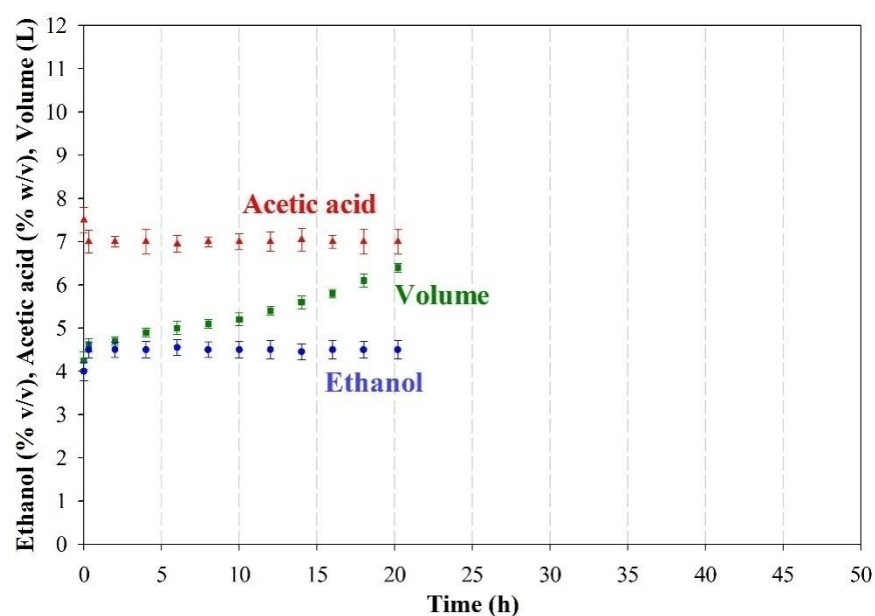
- Volume unloaded from the first bioreactor: 4.25 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 4.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 6.0 % (v/v).

- Temperature in the first bioreactor: 28 °C.
- Ethanol concentration during loading of the second bioreactor: 4.5 % (v/v).
- Temperature in the second bioreactor: 28 °C.

Figures S2.29 and S2.30 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of eight replications with their standard deviations.



**Figure S2.29.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 15.



**Figure S2.30.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 15.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.16 shows the values for Experiment 15.

**Table S2.16.** Experimental results obtained in Experiment 15



Variable	Value
Total cycle duration (h)	$20.3 \pm 0.6$
Duration of the fast loading stage in the first bioreactor (h)	$1.2 \pm 0.1$
Duration of the slow loading stage in the first bioreactor (h)	$12.3 \pm 0.4$
Duration of the depletion stage in the first bioreactor (h)	$6.8 \pm 0.7$
Duration of the fast loading stage in the second bioreactor (h)	$0.3 \pm 0.1$
Duration of the slow loading stage in the second bioreactor (h)	$19.9 \pm 0.5$
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	$0.059 \pm 0.005$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$0.606 \pm 0.027$
Time fraction of the depletion stage in the first bioreactor over the cycle duration	$0.335 \pm 0.037$
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	$0.015 \pm 0.005$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.985 \pm 0.035$
Mean volume in the fast loading stage in the first bioreactor (L)	$4.48 \pm 0.05$
Mean volume in the slow loading stage in the first bioreactor (L)	$6.60 \pm 0.05$
Mean volume in the depletion stage in the first bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the first bioreactor (L)	$6.94 \pm 0.35$
Mean volume in the fast loading stage in the second bioreactor (L)	$4.43 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$5.5 \pm 0.05$
Mean volume in the second bioreactor (L)	$5.48 \pm 0.22$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$12.43 \pm 0.41$
Volume of fermentation medium unloaded from the second reactor (L)	$6.40 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$4.5 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$5.5 \pm 0.2$
Mean ethanol concentration in the second bioreactor (% v/v)	$4.5 \pm 0.2$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$7.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$7.0 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$6.0 \pm 0.2$
Mean acetic acid concentration in the second bioreactor (% w/v)	$7.0 \pm 0.2$

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Mean rate of acetic acid formation in the first bioreactor (% w/v ·h <sup>-1</sup> )	0.23 ± 0.01
Mean rate of acetic acid formation in second bioreactor (% w/v ·h <sup>-1</sup> )	0.12 ± 0.01
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v ·h <sup>-1</sup> )	0.18 ± 0.01
Total acetic acid production in the first bioreactor (g acetic acid·h <sup>-1</sup> )	15.7 ± 0.6
Total acetic acid production in the second bioreactor (g acetic acid·h <sup>-1</sup> )	6.4 ± 0.8
Total acetic acid production in the two bioreactors as a whole (g acetic acid·h <sup>-1</sup> )	22.1 ± 0.9

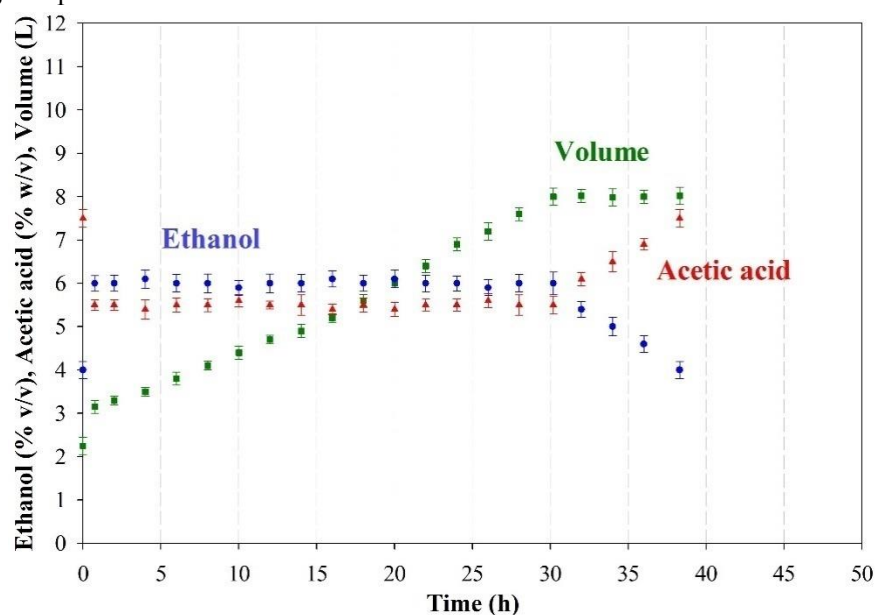
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## S2.16. Results of Experiment 16

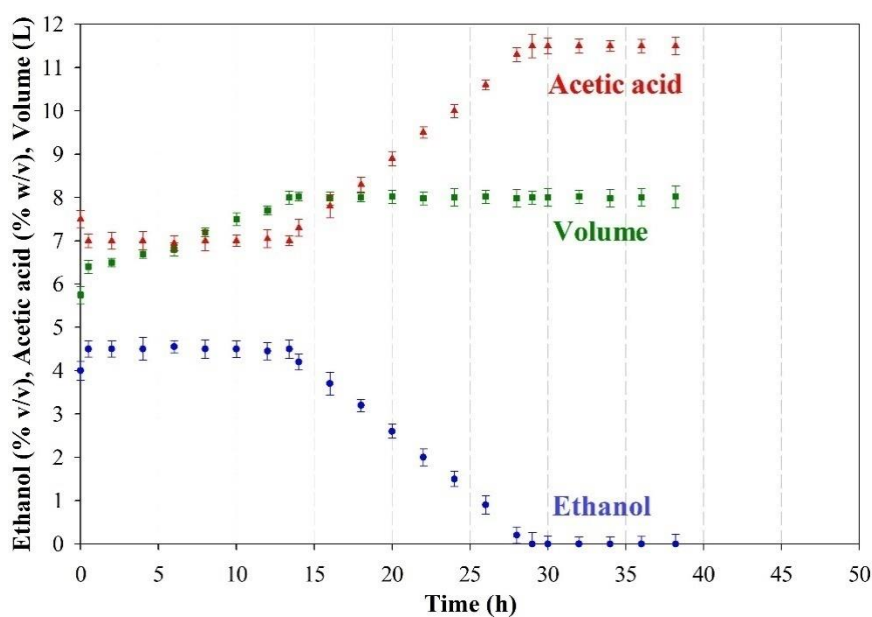
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 5.75 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 4.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 6.0 % (v/v).
- Temperature in the first bioreactor: 32 °C.
- Ethanol concentration during loading of the second bioreactor: 4.5 % (v/v).
- Temperature in the second bioreactor: 32 °C.

Figures S2.31 and S2.32 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of eight replications with their standard deviations.



**Figure S2.31.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 16.



**Figure S2.32.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 16.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.17 shows the values for Experiment 16.

**Table S2.17.** Experimental results obtained in Experiment 16

Variable	Value
Total cycle duration (h)	38.3 ± 0.6
Duration of the fast loading stage in the first bioreactor (h)	0.8 ± 0.1
Duration of the slow loading stage in the first bioreactor (h)	29.5 ± 0.4
Duration of the depletion stage in the first bioreactor (h)	8.1 ± 0.7
Duration of the fast loading stage in the second bioreactor (h)	0.5 ± 0.1
Duration of the slow loading stage in the second bioreactor (h)	12.9 ± 0.3
Duration of the depletion stage in the second bioreactor (h)	15.6 ± 0.6
Duration of starving in stage the second bioreactor (h)	9.2 ± 0.8
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	0.020 ± 0.003
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	0.769 ± 0.016
Time fraction of the depletion stage in the first bioreactor over the cycle duration	0.211 ± 0.019
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	0.013 ± 0.003
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	0.338 ± 0.010
Time fraction of the depletion stage in the second bioreactor over the cycle duration	0.408 ± 0.017
Time fraction of the starving stage in the second bioreactor over the cycle duration	0.241 ± 0.021
Mean volume in the fast loading stage in the first bioreactor (L)	2.70 ± 0.05
Mean volume in the slow loading stage in the first bioreactor (L)	5.58 ± 0.05
Mean volume in the depletion stage in the first bioreactor (L)	8.00 ± 0.05
Mean volume in the first bioreactor (L)	6.03 ± 0.18
Mean volume in the fast loading stage in the second bioreactor (L)	6.08 ± 0.05
Mean volume in the slow loading stage in the second bioreactor (L)	7.20 ± 0.05
Mean volume in the depletion stage in the second bioreactor (L)	8.00 ± 0.05
Mean volume in the starving stage in the second bioreactor (L)	8.00 ± 0.05
Mean volume in the second bioreactor (L)	7.70 ± 0.24
Mean overall volume during a cycle in the two bioreactors as a whole (L)	13.74 ± 0.30
Volume of fermentation medium unloaded from the second reactor (L)	8.00 ± 0.05
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	0.0 ± 0.2
Mean ethanol concentration in the first bioreactor (% v/v)	5.7 ± 0.1
Mean ethanol concentration in the second bioreactor (% v/v)	3.2 ± 0.4
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	7.5 ± 0.2
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	11.5 ± 0.2
Mean acetic acid concentration in the first bioreactor (% w/v)	5.8 ± 0.1
Mean acetic acid concentration in the second bioreactor (% w/v)	8.2 ± 0.4
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	0.19 ± 0.01
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	0.17 ± 0.01
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	0.17 ± 0.01
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	11.3 ± 0.3

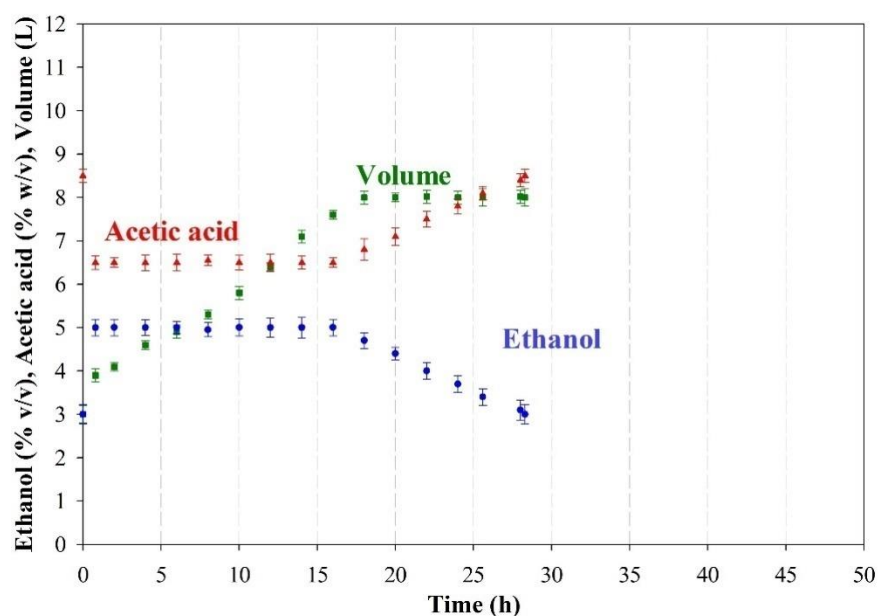
Total acetic acid production in the second bioreactor (g acetic acid·h <sup>-1</sup> )	12.8 ± 0.6
Total acetic acid production in the two bioreactors as a whole (g acetic acid·h <sup>-1</sup> )	24.0 ± 0.6

### S2.17. Results of Experiment 17

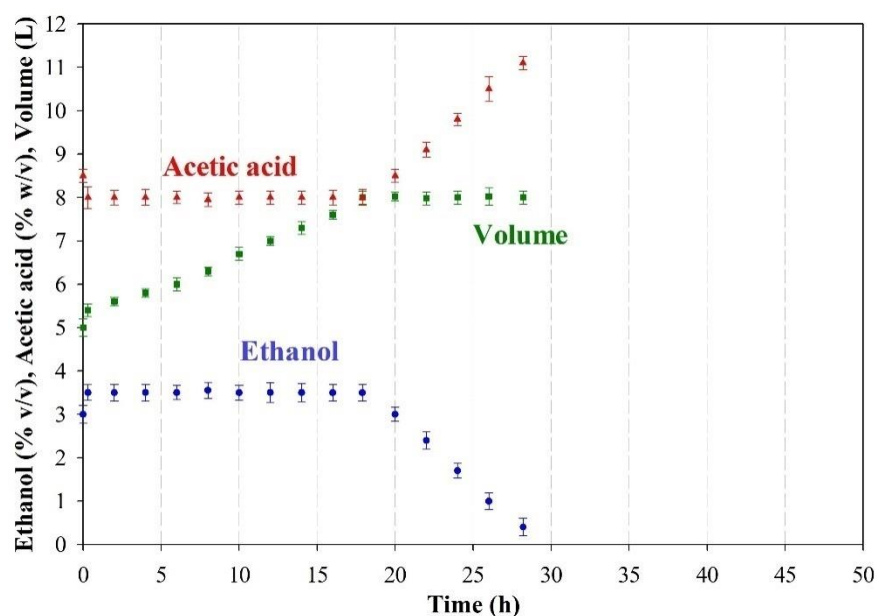
The operating conditions used in this experiment were as follows:

- Volume unloaded from the first bioreactor: 5.00 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 3.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 5.0 % (v/v).
- Temperature in the first bioreactor: 30 °C.
- Ethanol concentration during loading of the second bioreactor: 3.5 % (v/v).
- Temperature in the second bioreactor: 30 °C.

Figures S2.33 and S2.34 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of nine replications with their standard deviations.



**Figure S2.33.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 17.



**Figure S2.34.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 17.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.18 shows the values for Experiment 17.

**Table S2.18.** Experimental results obtained in Experiment 17

Variable	Value
Total cycle duration (h)	$28.3 \pm 0.7$
Duration of the fast loading stage in the first bioreactor (h)	$0.8 \pm 0.1$
Duration of the slow loading stage in the first bioreactor (h)	$15.2 \pm 0.3$
Duration of the depletion stage in the first bioreactor (h)	$12.3 \pm 0.8$
Duration of the fast loading stage in the second bioreactor (h)	$0.3 \pm 0.1$
Duration of the slow loading stage in the second bioreactor (h)	$17.6 \pm 0.3$
Duration of the depletion stage in the second bioreactor (h)	$10.3 \pm 0.7$
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	$0.028 \pm 0.004$
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	$0.537 \pm 0.017$
Time fraction of the depletion stage in the first bioreactor over the cycle duration	$0.435 \pm 0.029$
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	$0.011 \pm 0.004$
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	$0.624 \pm 0.017$
Time fraction of the depletion stage in the second bioreactor over the cycle duration	$0.365 \pm 0.025$
Mean volume in the fast loading stage in the first bioreactor (L)	$3.45 \pm 0.05$

Mean volume in the slow loading stage in the first bioreactor (L)	$5.95 \pm 0.05$
Mean volume in the depletion stage in the first bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the first bioreactor (L)	$6.77 \pm 0.25$
Mean volume in the fast loading stage in the second bioreactor (L)	$5.20 \pm 0.05$
Mean volume in the slow loading stage in the second bioreactor (L)	$6.70 \pm 0.05$
Mean volume in the depletion stage in the second bioreactor (L)	$8.00 \pm 0.05$
Mean volume in the second bioreactor (L)	$7.16 \pm 0.25$
Mean overall volume during a cycle in the two bioreactors as a whole (L)	$13.93 \pm 0.35$
Volume of fermentation medium unloaded from the second reactor (L)	$8.00 \pm 0.05$
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	$0.4 \pm 0.2$
Mean ethanol concentration in the first bioreactor (% v/v)	$4.7 \pm 0.2$
Mean ethanol concentration in the second bioreactor (% v/v)	$2.9 \pm 0.3$
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	$8.5 \pm 0.2$
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	$11.1 \pm 0.2$
Mean acetic acid concentration in the first bioreactor (% w/v)	$6.8 \pm 0.2$
Mean acetic acid concentration in the second bioreactor (% w/v)	$8.6 \pm 0.3$
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	$0.22 \pm 0.01$
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	$0.23 \pm 0.01$
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	$0.23 \pm 0.01$
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	$15.0 \pm 0.5$
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	$16.4 \pm 0.8$
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	$31.4 \pm 1.0$

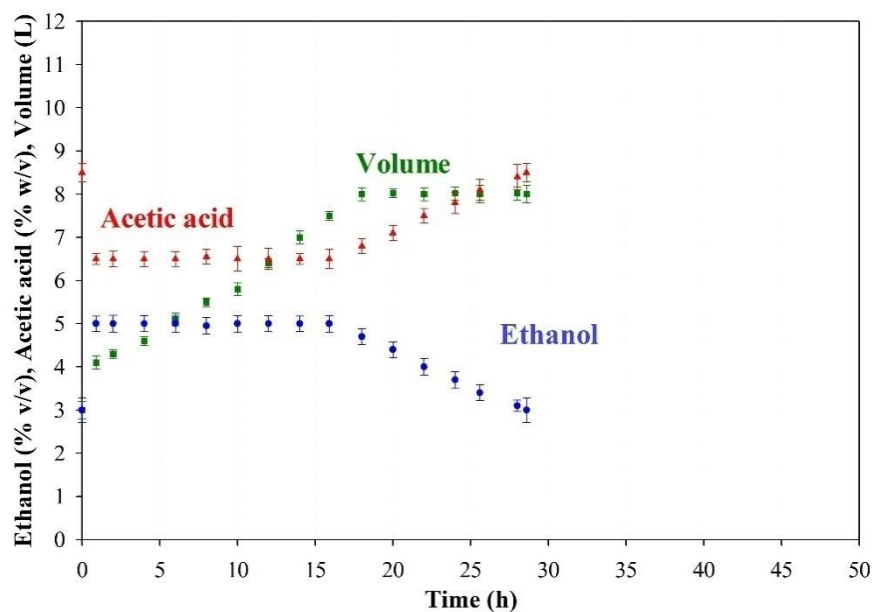
## S2.18. Results of Experiment 18

The operating conditions used in this experiment were as follows:

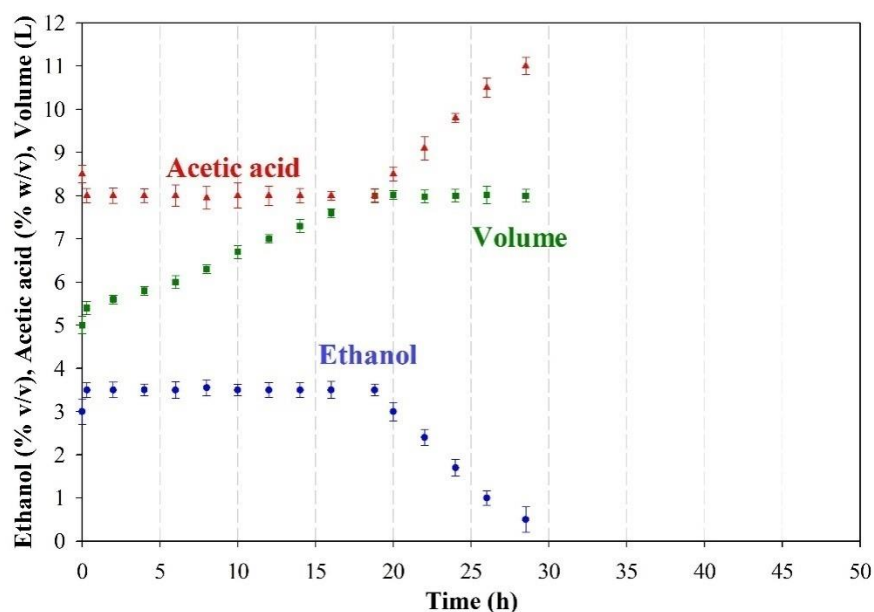
- Volume unloaded from the first bioreactor: 5.00 L.
- Total ethanol concentration at the time the first bioreactor was unloaded: 3.0 % (v/v).
- Ethanol concentration during loading of the first bioreactor: 5.0 % (v/v).
- Temperature in the first bioreactor: 30 °C.
- Ethanol concentration during loading of the second bioreactor: 3.5 % (v/v).

- Temperature in the second bioreactor: 30 °C.

Figures S2.35 and S2.36 show the variation of the ethanol concentration, acidity, volume, and total and viable cells, in the first and second bioreactor, respectively. All values shown are the means of nineteen replications with their standard deviations.



**Figure S2.35.** Time course of the ethanol concentration, acidity and volume of medium in the first bioreactor in Experiment 18.



**Figure S2.36.** Time course of the ethanol concentration, acidity and volume of medium in the second bioreactor in Experiment 18.

The procedure used to obtain the different variables is described in detail in Supplementary Material 1. By way of summary, Table S2.19 shows the values for Experiment 18.

**Table S2.19.** Experimental results obtained in Experiment 18

Variable	Value
Total cycle duration (h)	28.6 ± 0.8



Duration of the fast loading stage in the first bioreactor (h)	0.9 ± 0.1
Duration of the slow loading stage in the first bioreactor (h)	15.0 ± 0.2
Duration of the depletion stage in the first bioreactor (h)	12.7 ± 0.8
Duration of the fast loading stage in the second bioreactor (h)	0.3 ± 0.1
Duration of the slow loading stage in the second bioreactor (h)	18.5 ± 0.4
Duration of the depletion stage in the second bioreactor (h)	9.7 ± 0.9
Time fraction of the fast loading stage in the first bioreactor over the cycle duration	0.031 ± 0.004
Time fraction of the slow loading stage in the first bioreactor over the cycle duration	0.524 ± 0.017
Time fraction of the depletion stage in the first bioreactor over the cycle duration	0.444 ± 0.031
Time fraction of the fast loading stage in the second bioreactor over the cycle duration	0.011 ± 0.004
Time fraction of the slow loading stage in the second bioreactor over the cycle duration	0.649 ± 0.023
Time fraction of the depletion stage in the second bioreactor over the cycle duration	0.340 ± 0.033
Mean volume in the fast loading stage in the first bioreactor (L)	3.55 ± 0.05
Mean volume in the slow loading stage in the first bioreactor (L)	6.05 ± 0.05
Mean volume in the depletion stage in the first bioreactor (L)	8.00 ± 0.05
Mean volume in the first bioreactor (L)	6.84 ± 0.27
Mean volume in the fast loading stage in the second bioreactor (L)	5.20 ± 0.05
Mean volume in the slow loading stage in the second bioreactor (L)	6.70 ± 0.05
Mean volume in the depletion stage in the second bioreactor (L)	8.00 ± 0.05
Mean volume in the second bioreactor (L)	7.13 ± 0.32
Mean overall volume during a cycle in the two bioreactors as a whole (L)	13.96 ± 0.42
Volume of fermentation medium unloaded from the second reactor (L)	8.00 ± 0.05
Final ethanol concentration at the time the second bioreactor was unloaded (% v/v)	0.5 ± 0.2
Mean ethanol concentration in the first bioreactor (% v/v)	4.7 ± 0.2
Mean ethanol concentration in the second bioreactor (% v/v)	2.9 ± 0.2
Final acetic acid concentration at the time the first bioreactor was unloaded (% w/v)	8.5 ± 0.2
Final acetic acid concentration at the time the second bioreactor was unloaded (% w/v)	11.0 ± 0.2
Mean acetic acid concentration in the first bioreactor (% w/v)	6.8 ± 0.2
Mean acetic acid concentration in the second bioreactor (% w/v)	8.6 ± 0.2
Mean rate of acetic acid formation in the first bioreactor (% w/v · h <sup>-1</sup> )	0.22 ± 0.01
Mean rate of acetic acid formation in second bioreactor (% w/v · h <sup>-1</sup> )	0.22 ± 0.01
Mean overall rate of acetic acid formation in the two bioreactors as a whole (% w/v · h <sup>-1</sup> )	0.22 ± 0.01
Total acetic acid production in the first bioreactor (g acetic acid · h <sup>-1</sup> )	14.9 ± 0.5
Total acetic acid production in the second bioreactor (g acetic acid · h <sup>-1</sup> )	16.0 ± 0.8
Total acetic acid production in the two bioreactors as a whole (g acetic acid · h <sup>-1</sup> )	30.8 ± 1.0

