applied sciences

MDPI

Supplementary Material 1

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

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S1. Calculating selected target variables

Below is explained the procedure used to calculate:

- The rate of acetic acid formation.
- The acetic acid production.
- The mean overall volume for a cycle.

Figures S1.1, S1.2 and S1.3 show the profiles for typical experiments. The profiles for the second bioreactor are twofold because, depending on the particular aim, the reactor will require prior depletion to the preset ethanol concentration during the loading or fast loading stage. The operating conditions for each experiment will be dictated by the values of the following variables:

- Volume unloaded from the first bioreactor (V_{u1}, L).
- Ethanol concentration at the time the first bioreactor is unloaded (E_{u1}, % v/v).
- Ethanol concentration during loading of the first bioreactor (E_{l1}, % v/v).
- Temperature in the first bioreactor (T_1, °C).
- Ethanol concentration during loading of the second bioreactor (E_{l2}, % v/v).
- Temperature in the second bioreactor (T_2, °C).

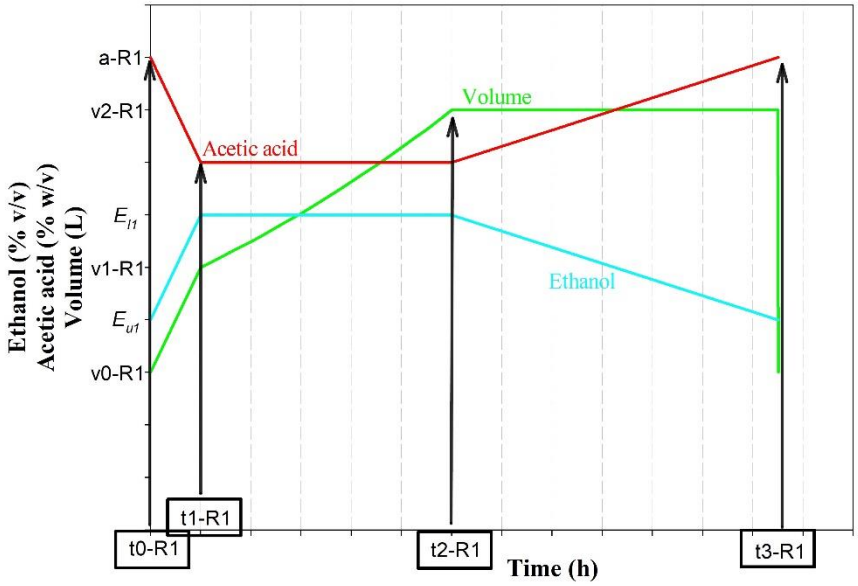

Figure S1.1. Time course of the ethanol concentration, acidity and volume in the first reactor in a typical experiment.

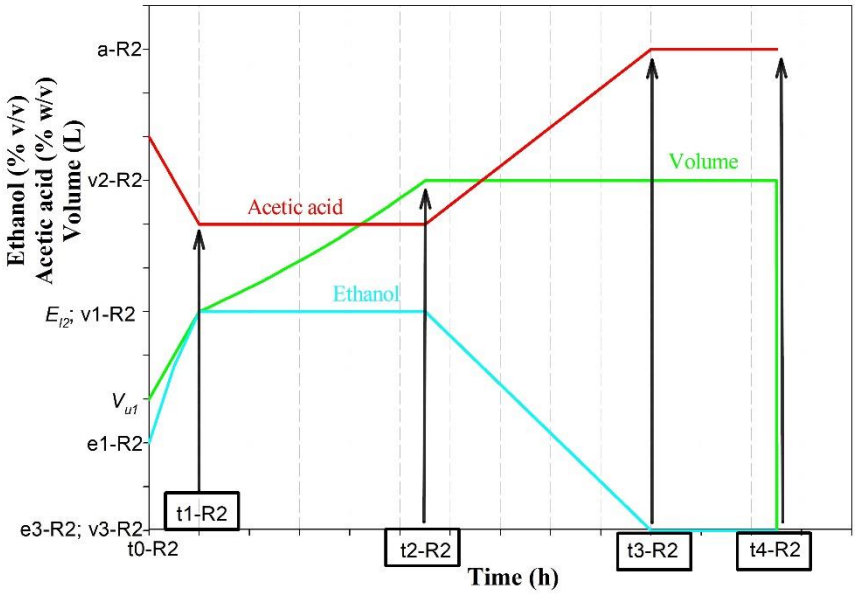

Figure S1.2. Time course of the ethanol concentration, acidity and volume in the second reactor in a typical experiment involving a fast loading stage.

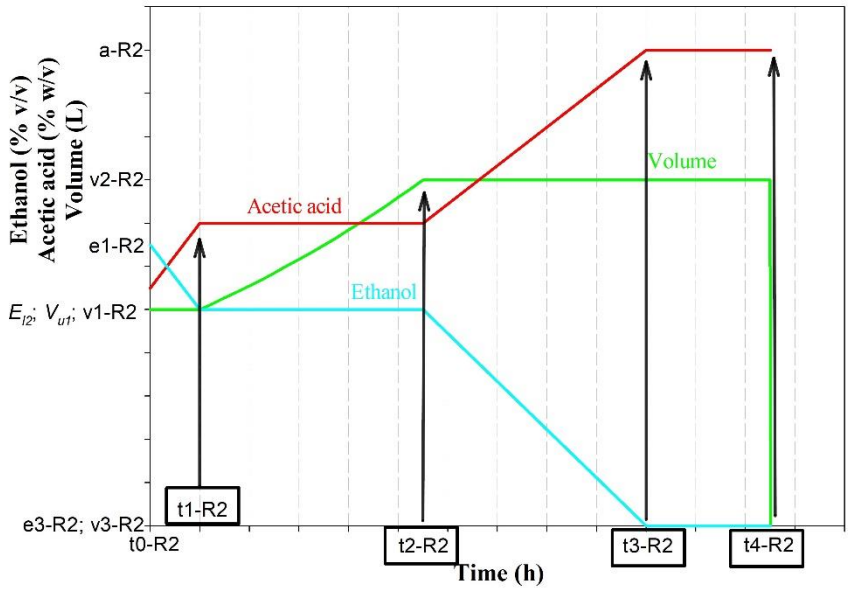

Figure S1.3. Time course of the ethanol concentration, acidity and volume in the second reactor in a typical experiment involving a previous depletion stage.

Figure S1.1 shows three distinct zones in the profile for the first bioreactor, namely:

- An initial zone corresponding to the fast loading stage spanning the time until t_1-R_1 (h), when the bioreactor is rapidly loaded at a given rate until an ethanol concentration E_{l1} (% v/v) and a volume v_1-R_1 (L) are reached. This loading operation dilutes the medium, thereby decreasing the acid concentration and increasing the ethanol concentration.
- A central zone corresponding to the slow loading step that spans the time from t_1-R_1 (h) to t_2-R_1 (h) and where the ethanol concentration oscillates slightly but remains essentially constant at E_{l1} (% v/v) by effect of the semi-continuous addition of wine to replenish ethanol used by bacteria and/or swept with volatiles until the maximum volume (v_2-R_1, L) is reached.
- After the maximum volume is reached, a third zone corresponding to depletion is observed that occurs through the end of a cycle (t_3-R_1, h). In this stage, the ethanol concentration is allowed to decrease to E_{u1} (% v/v) before the preset volume of medium (v_2-R_1 – v_0-R_1, L) is unloaded.

It should be noted that the duration of a cycle is identical in both bioreactors. Such a duration is equal to the sum of lengths of the previous three zones for the first reactor and of the following four stages in the second. In fact, Figs S1.2 and S1.3 show four distinct zones for the second bioreactor:

- Depending on whether e_1-R_2 is greater or less than E_{l2}, the graph starts with a zone of pre-depletion or fast loading. This zone spans the time from the start of a cycle to t_1-R_2 (h). If e_1-$R_2 > E_{l2}$, the zone corresponds to a pre-depletion step where the ethanol concentration decreases from e_1-R_2 to E_{l2} (% v/v) (viz., the preset concentration for the loading stage) before fresh wine is added to the medium. On the other hand, if e_1-$R_2 < E_{l2}$, the zone corresponds to a fast loading step where the reactor is continuously loaded at a high rate to an ethanol concentration E_{l2} (% v/v) and a volume v_1-R_2 (L).
- There follows a second, slow loading stage spanning the times from t_1-R_2 (h) to t_2-R_2 (h) where the ethanol concentration oscillates slightly but essentially remains constant at E_{l2} (% v/v) by effect of the semi-continuous addition of the amount of wine needed to replenish ethanol used by the bacteria or swept with other volatiles until the maximum volume (v_2-R_2, L) is reached.
- A third, depletion stage then starts that lasts until ethanol in the medium is fully depleted at t_3-R_2.
- In the last, fourth stage, all ethanol has previously been depleted but the cycle in the first reactor continues to develop until t_4-R_2 (h). When the ethanol concentration in the first bioreactor falls to E_{u1} (% v/v), the whole volume of medium in the second is unloaded and then a volume (v_2-R_1 – v_0-R_1, L) (i.e., V_{u1}, L) is unloaded from the first into the second.

Below is described the procedure used to calculate the fermentation rates from acidity data [6].

S1.1 Rate of acetic acid formation in the first bioreactor

The mean rate of acetic acid formation can be calculated from the following equation:

$$(r_A)_{mR1} = \frac{HAc_{end\ R1} \cdot V_{u1}}{t_{cycle} \cdot V_{mediumR1}}, \quad \text{(S1.1)}$$

where:
$(r_A)_{mR1}$: mean rate of acetic acid formation in the first bioreactor (% w/v ·h^{-1}).
$HAc_{end\ R1}$: final acetic acid concentration at the time the first bioreactor is unloaded (% w/v).
V_{d1}: volume of fermentation medium unloaded from the first reactor (L).
t_{cycle}: duration of a cycle (h).
$V_{mediumR1}$: mean volume of medium in the first bioreactor during a cycle (L).

Obtaining $V_{mediumR1}$ requires calculating the volume of medium for each stage and the fraction of time over the duration of a cycle.

In the fast loading stage,

$$\tilde{V}_{FL1} = \frac{v_{FL1} + V_{01}}{2}, \quad \text{(S1.2)}$$

where:
$\tilde{V}_{FL1}$: mean volume of medium during the fast loading stage in the first bioreactor (L).
v_{FL1}: volume at the end of the fast loading stage in the first bioreactor (L).
V_{01}: initial volume in the first bioreactor (L).

In the slow loading stage,

$$\tilde{V}_{SL1} = \frac{v_{SL1} + v_{FL1}}{2}, \quad \text{(S1.3)}$$

where:
$\tilde{V}_{SL1}$: mean volume of medium during the slow loading stage in the first bioreactor (L).
v_{SL1}: volume at the end of the slow loading stage in the first bioreactor (L).

In the depletion stage,

$$\tilde{V}_{D1} = \frac{v_{D1}+v_{SL1}}{2}, \quad \text{(S1.4)}$$

where:
$\tilde{V}_{D1}$: mean volume of medium during the depletion stage in the first bioreactor (L).
v_{D1}: final volume in the depletion stage in the first bioreactor (L).

The previous mean volumes are now used to obtain the mean for a cycle with provision for the fraction of time corresponding to each stage:

$$V_{mediumR1} = T_{FL1} \cdot \tilde{V}_{FL1} + T_{SL1} \cdot \tilde{V}_{SL1} + T_{D1} \cdot \tilde{V}_{D1}, \quad \text{(S1.5)}$$

T_{FL1} is given by

$$T_{FL1} = \frac{t_{FL1}}{t_T}, \quad \text{(S1.6)}$$

where:
T_{FL1}: fraction of time of the fast loading stage in the first bioreactor over the cycle duration.
t_{FL1}: duration of the fast loading stage in the first bioreactor (h).

On the other hand, T_{SL1} is given by

$$T_{SL1} = \frac{t_{SL1}}{t_{cycle}}, \quad \text{(S1.7)}$$

where:
T_{SL1}: fraction of time of the slow loading stage in the first bioreactor over the cycle duration.
t_{SL1}: duration of the slow loading stage in the first bioreactor (h).

Finally, T_{D1} is given by

$$T_{D1} = \frac{t_{D1}}{t_T}, \quad \text{(S1.8)}$$

where:
T_{D1}: fraction of time of the depletion stage in the first bioreactor over the cycle duration.
t_{D1}: duration of the depletion stage in the first bioreactor (h).

Once the mean volume has been obtained, it can be calculated the mean acetification rate of the process from eq. S1.1.

S1.2. Rate of acetic acid formation in the second bioreactor

Similarly to $(r_A)_{mR1}$, the rate of acetic acid formation in the second reactor can be calculated from the following equation:

$$(r_A)_{mR2} = \frac{HAc_{end\ R2} \cdot V_{u2} - HAc_{initialR2} \cdot V_{02}}{t_{cycle} \cdot V_{mediumR2}}, \quad \text{(S1.9)}$$

where:
$(r_A)_{mR2}$: mean rate of acetic acid formation in the second bioreactor (% w/v ·h^{-1}).
$HAc_{end\ R2}$: final acetic acid concentration in the second bioreactor (% w/v).
V_{u2}: volume of fermentation medium unloaded from the second bioreactor (L).
$HAc_{initialR2}$: initial concentration of acetic acid in the second bioreactor in a cycle (% w/v).
V_{02}: initial volume in the second bioreactor (L).
$V_{mediumR2}$: mean volume of fermentation medium in the second bioreactor during a cycle (L).

Similarly to the first bioreactor, one must calculate the mean volume of medium in the second for each stage in a cycle.

In the pre-depletion stage,

$$\tilde{V}_{PD2} = \frac{v_{PD2}+V_{02}}{2}, \quad \text{(S1.10)}$$

where:
$\tilde{V}_{PD2}$: mean volume of medium in the second bioreactor during the pre-depletion stage (L).
v_{PD2}: volume of medium in the second bioreactor at the end of the pre-depletion stage (L).
V_{02}: initial volume in the second bioreactor (L).

In the fast loading stage,

$$\tilde{V}_{FL2} = \frac{v_{FL2}+V_{02}}{2}, \quad \text{(S1.11)}$$

where:
$\tilde{V}_{FL2}$: mean volume of medium in the second bioreactor during the fast loading stage (L).
v_{FL2}: final volume in the second bioreactor in the fast loading stage (L).
V_{02}: initial volume in the second bioreactor (L).

In the slow loading stage,

$$\tilde{V}_{SL2} = \frac{v_{SL2}+v_{FL2}}{2}, \quad \text{(S1.12)}$$

where:
$\tilde{V}_{SL2}$: mean volume of medium in the second bioreactor during the slow loading stage (L).
v_{SL2}: final volume in the second bioreactor in the slow loading stage (L).

Finally, in the depletion stage,

$$\tilde{V}_{D2} = \frac{v_{D2}+v_{SL2}}{2}, \quad \text{(S1.13)}$$

where:
$\tilde{V}_{D2}$: mean volume of medium in the second bioreactor during the depletion stage (L).
v_{D2}: final volume in the second bioreactor in the depletion stage (L).

The previous mean volumes can be used to calculate the mean volume for a cycle with provision for the fraction of time taken by of each stage.

$$V_{mediumR2} = T_{PD2} \cdot \tilde{V}_{PD2} + T_{FL2} \cdot \tilde{V}_{FL2} + T_{SL2} \cdot \tilde{V}_{SL2} + T_{D2} \cdot \tilde{V}_{D2}, \quad \text{(S1.14)}$$

In this equation,

$$T_{PD2} = \frac{t_{PD2}}{t_T}, \quad \text{(S1.15)}$$

where:
T_{PD2}: fraction of time of the pre-depletion stage in the second bioreactor over the cycle duration.
t_{PD2}: duration of the pre-depletion stage in the second bioreactor (h).

Similarly to T_{PD2}, T_{FL2} in eq. S1.14 is given by

$$T_{FL2} = \frac{t_{FL2}}{t_T}, \quad \text{(S1.16)}$$

where:
T_{FL2}: fraction of time of the fast loading stage in the second bioreactor over the cycle duration.
t_{FL2}: duration of the fast loading stage in the second bioreactor (h).

T_{SL2} in eq. S1.14 is given by

$$T_{SL2} = \frac{t_{SL2}}{t_{cycle}}, \quad \text{(S1.17)}$$

where:
T_{SL2}: fraction of time of the slow loading stage in the second bioreactor over the cycle duration.
t_{SL2}: duration of the slow loading stage in the second bioreactor (h).

Finally, T_{D2} is defined as

$$T_{D2} = \frac{t_{D2}}{t_T}, \quad \text{(S1.18)}$$

where:
T_{D2}: fraction of time of the depletion stage in the second bioreactor over the cycle duration.
t_{D2}: duration of the depletion stage in the second bioreactor (h).

Once the mean reaction volume is obtained, it can be used to calculate the mean acetification rate of the process in the second reactor from eq. S1.9.

S1.3. Overall rate of acetic acid formation in the two-bioreactor system

Obtaining the overall rate of acetic acid formation requires the prior calculation of the overall volume of medium during a cycle, which will be the combination of those in the two reactors:

$$V_m = V_{mediumR1} + V_{mediumR2}, \quad \text{(S1.19)}$$

where:
V_m: overall volume of medium during a cycle (L), which is used to calculate the rate of acetic acid formation:

$$(r_A)_{global} = \frac{HAc_{end\,R2} \cdot V_{d2}}{t_{cycle} \cdot V_m}, \quad \text{(S1.20)}$$

S1.4. Total acetic acid production in the two-bioreactor system

The total production of acetic acid, which is of especial industrial interest, can be calculated from the following equation:

$$P_m = \frac{10 \cdot HAc_{end\,R2} \cdot V_{d2}}{t_{cycle}}, \quad \text{(S1.21)}$$

where:
P_m: total acetic acid production (g acetic acid·h^{-1}).

However, assessing the feasibility of operating in alternative ways requires determining the extent to which each bioreactor contributes to the total acid production as explained below.

S1.5. Acetic acid production in the first bioreactor

Acetic acid production in the first reactor is calculated from the following equation:

$$P_{A_{R1}} = \frac{10 \cdot HAc_{end\,R1} \cdot V_{d1}}{t_{cycle}}, \quad \text{(S1.22)}$$

S1.6. Acetic acid production in the second bioreactor

Calculating the rate of acid production in the second bioreactor requires considering the acidity of the inoculum from the first bioreactor, which entails altering the previous equations:

$$P_{A_{R2}} = \frac{10 \cdot (HAc_{finalR2} \cdot V_{d2} - HAc_{initialR2} \cdot V_{02})}{t_{cycle}}, \quad \text{(S1.23)}$$

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).
E_{u1}: ethanol concentration at the time the first reactor was unloaded (% v/v).
$HAc_{end\ R1}$: final acetic acid concentration at the time the first bioreactor is unloaded (% w/v).
$HAc_{end\ R2}$: final acetic acid concentration in the second bioreactor (% w/v).
$HAc_{initialR2}$: initial concentration of acetic acid in the second bioreactor in a cycle (% w/v).
P_m: 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}).
$(r_A)_{mR1}$: mean rate of acetic acid formation in the first bioreactor (% w/v ·h^{-1}).
$(r_A)_{mR2}$: mean rate of acetic acid formation in the second bioreactor (% w/v ·h^{-1}).
T_1: temperature in the first reactor (ºC).
T_2: temperature in the second reactor (ºC).
t_{cycle}: duration of a cycle (h).
T_{D1}: fraction of time of the depletion stage in the first bioreactor over the cycle duration.
T_{D2}: fraction of time of the depletion stage in the second bioreactor over the cycle duration.
t_{D1}: duration of the depletion stage in the first bioreactor (h).
t_{D2}: duration of the depletion stage in the second bioreactor (h).
T_{FL1}: fraction of time of the fast loading stage in the first bioreactor over the cycle duration.
T_{FL2}: fraction of time of the fast loading stage in the second bioreactor over the cycle duration.
t_{FL1}: duration of the fast loading stage in the first bioreactor (h).
t_{FL2}: duration of the fast loading stage in the second bioreactor (h).
T_{PD2}: fraction of time of the pre-depletion stage in the second bioreactor over the cycle duration.
t_{PD2}: duration of the pre-depletion stage in the second bioreactor (h).
T_{SL1}: fraction of time of the slow loading stage in the first bioreactor over the cycle duration.
T_{SL2}: fraction of time of the slow loading stage in the second bioreactor over the cycle duration.
t_{SL1}: duration of the slow loading stage in the first bioreactor (h).
t_{SL2}: duration of the slow loading stage in the second bioreactor (h).
V_{01}: initial volume in the first bioreactor (L).
V_{02}: initial volume in the second bioreactor (L).
V_{d1}: volume of fermentation medium unloaded from the first reactor (L).
$\tilde{V}_{D1}$: mean volume of medium during the depletion stage in the first bioreactor (L).
$\tilde{V}_{D2}$: mean volume of medium in the second bioreactor during the depletion stage (L).
v_{D1}: final volume in the depletion stage in the first bioreactor (L).
v_{D2}: final volume in the second bioreactor in the depletion stage (L).
$\tilde{V}_{FL1}$: mean volume of medium during the fast loading stage in the first bioreactor (L).
$\tilde{V}_{FL2}$: mean volume of medium in the second bioreactor during the fast loading stage (L).
v_{FL1}: volume at the end of the fast loading stage in the first bioreactor (L).
v_{FL2}: final volume in the second bioreactor in the fast loading stage (L).
V_m: overall volume of medium during a cycle (L).
$V_{mediumR1}$: mean volume of medium in the first bioreactor during a cycle (L).
$V_{mediumR2}$: mean volume of fermentation medium in the second bioreactor during a cycle (L).
$\tilde{V}_{PD2}$: mean volume of medium in the second bioreactor during the pre-depletion stage (L).
v_{PD2}: volume of medium in the second bioreactor at the end of the pre-depletion stage (L).
$\tilde{V}_{SL1}$: mean volume of medium during the slow loading stage in the first bioreactor (L).
$\tilde{V}_{SL2}$: mean volume of medium in the second bioreactor during the slow loading stage (L).
v_{SL1}: volume at the end of the slow loading stage in the first bioreactor (L)
v_{SL2}: final volume in the second bioreactor in the slow loading stage (L).

V_{u1}: volume unloaded from the first reactor (L).
V_{u2}: volume of fermentation medium unloaded from the second bioreactor (L).