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Estimating the mean acetification rate via on-line monitored changes in ethanol during a semi-continuous vinegar production cycle

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Abstract

A simple, fast method for estimating the mean rate of biological oxidation in a typical semi-continuous wine vinegar production cycle was developed. The method involves on-line monitoring changes in ethanol concentration during the cycle and allows the acetification rate in each cycle step to be estimated. This datum is of a high interest with a view to optimizing the process by establishing the influence of the operational variables on productivity.

Keywords: Vinegar; Wine; Acetification rate

1. Introduction

Wine vinegar is industrially produced largely by using a semi-continuous process where a fraction of the total volume of a submerged culture is withdrawn on a periodic basis (Arnold, Becker, Delgado, Emde, & Enenkel, 2002; Ebner & Follmann, 1983; Levonen & Llaguno, 1983; Llaguno Marchena & Polo, 1991; Suarez Lepe & Iñigo Leal, 1992; Tesfaye, Morales, García-Parrilla, & Troncoso, 2002). The remaining volume, which acts as an inoculum for the next cycle, is replenished with fresh wine that is added in small portions to obtain the final working volume. This procedure results in temporal changes in the properties of the culture medium where acetic bacteria grow.

Industrial acetification processes are usually conducted in reactors of the self-aspirating turbine type. The high efficiency with which oxygen is transferred from the air to the culture medium results in a low energy consumption per litre of converted ethanol (Ebner, Sellmer, & Follmann, 1995).

The acetification process can be performed in various ways. Thus, depending on the time the reactor is unloaded, the unloaded volume and the way the reactor is loaded, among other factors, the culture medium can undergo more of less abrupt changes that may alter the concentration and activity of the bacterial population it contains. Optimizing the process entails examining the influence of diverse factors on the reactor performance. This involves much experimental work (Garrido-Vidal, Pizarro, & González-Sáiz, 2003) owing to the large number of variables potentially affecting the process and the typical difficulty encountered in experiments with microbial reactions.

Specifically, one must accurately determine the overall acetification rate in each semi-continuous work cycle in order to compare it with values obtained under different experimental conditions. The overall acetification rate is usually determined from the final acidity of the culture medium immediately prior to unloading the reactor, the

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unloaded volume and the total duration of the fermentation cycle. However, the rate can – and should – also be determined from the variation of the ethanol concentration during a cycle. A number of devices for continuously monitoring the ethanol concentration in the culture medium (e.g. Alkosens and Frings probes) are currently available.

Effectively optimizing vinegar production entails determining the acetification rate in both ways. Its determination from the ethanol concentration allows one to establish the variation of the biological oxidation rate throughout the cycle, which is especially interesting with a view to assessing the influence of operational variables on the different steps of the process and alter them as required to improve the outcome. On the other hand, determining the acetification rate from final acidity data provides an additional measure of the amount of substrate evaporated through aeration in each cycle as well as other forms of ethanol consumptions. In any case, it is known that different ethanol consumptions to that used for acetic acid formation are very small and can be directly neglected (Gómez & Cantero, 1998; González-Sáiz, Pizarro, & Garrido-Vidal, 2003; Garrido-Vidal et al., 2003).

In this work, we developed a simple, fast method for estimating the acetification rate from the variation of the ethanol concentration over a typical wine acetification cycle.

2. Material and methods

2.1. Microorganisms

The inoculum used consisted of a mixed culture of *Acetobacter* and *Gluconobacter* bacteria that were obtained from a fully operational industrial fermentation tank. The total cell concentration during a cycle ranged from 3×10^8 to 4×10^8 cell mL^{-1}.

2.2. Substrate

The substrate employed was white wine from the Montilla-Moriles region (protected designation of origin in southern Spain) with an ethanol content of 12 °GL ± 0.5 (°GL = mL ethanol/100 mL of medium) and an initial acidity of 0.2 °Acetic acid (°Acetic acid = g acetic acid/100 mL of medium).

2.3. Fermentation conditions

Experiments were conducted in a Frings 8 L fermentation tank, following a procedure mimicking those employed at many industrial plants and involving the following:

(1) Setting a constant temperature of 31 °C and allowing the reaction to proceed to an ethanol concentration of ca. 1.5 °GL and then rapidly unloading 50% of the tank contents.
(2) Slowly refilling the tank to the final working volume without exceeding a preset ethanol concentration (e.g. 5 °GL) at any time. This entails initially loading the tank in a continuous manner to the desired ethanol concentration and then, in a semi-continuous manner, to the final working volume (8 L in our case) without exceeding the aforesaid ethanol concentration.
(3) Passing air at a flow-rate of 7.5 L per litre of medium per hour.

The bioreactor was operated in an automated manner and was loaded, unloaded, controlled and monitored without operator intervention via previously programmed computer software.

This operational procedure resulted in temporal changes in some operational variables including volume, ethanol concentration, acidity and cell concentration.

3. Results and discussion

Fig. 1 shows the results obtained in a typical acetification cycle performed as described in the previous section. The graph shows three distinct regions, namely:

(a) One spanning the period where the reactor was continuous loaded to a 5 °GL ethanol concentration.
(b) A second one where the ethanol concentration remained constant by effect of the semi-continuous addition of wine to replenish the ethanol previously used by the bacteria.
(c) A final region where, once the maximum preset culture volume was reached, the concentration of ethanol decreased in a sustained manner until the reactor was unloaded.

Fig. 2 shows the variation of the ethanol concentration and volume of the medium in the first region. The bold line represents the variation of such a concentration as esti-

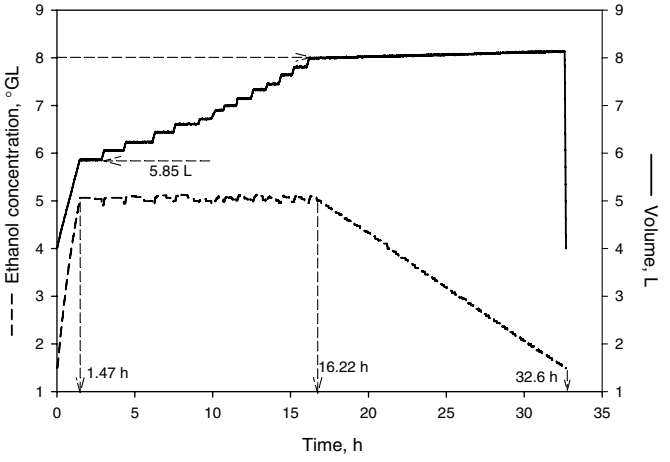

Fig. 1. Variation of the ethanol concentration and volume during a typical wine acetification cycle.

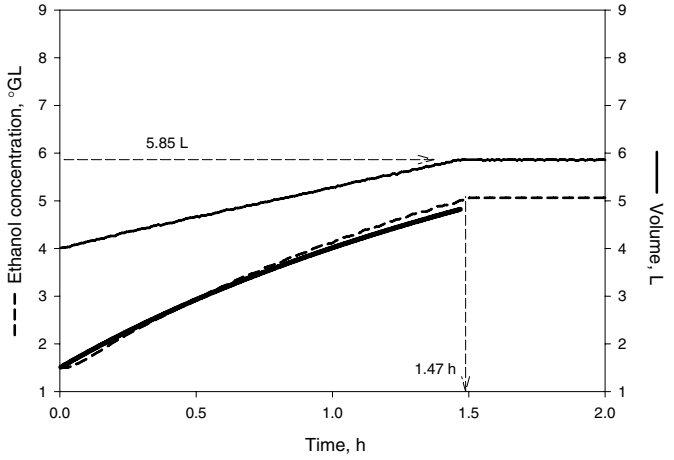

Fig. 2. Variation of experimental and estimated (bold line) ethanol concentration as well as volume during the first loading stage in a typical wine acetification cycle.

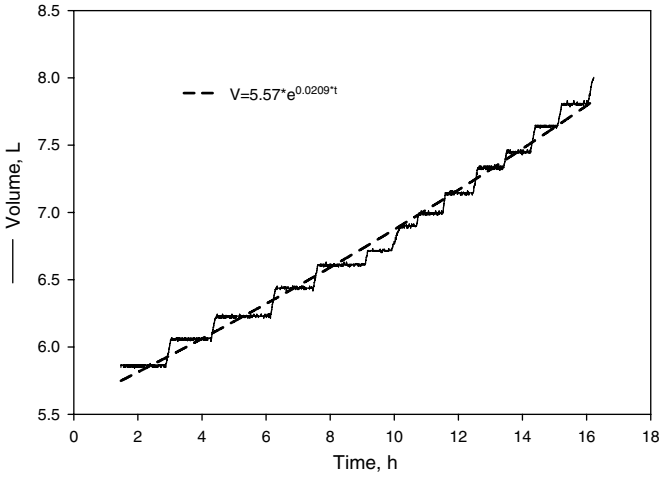

Fig. 3. Variation of experimental and fitted (discontinuous line) volume during the second loading step.

mated from a mass balance ignoring the bacterial uptake of ethanol. Obviously, the deviation of the experimental curve from the theoretical one provides a measure of the amount of ethanol used and/or that entrained through aeration of the reactor. As can be seen, the differences were virtually negligible, and so were the bacterial uptake of ethanol and the amount of alcohol entrained as a result.

The mass balance performed relied on the assumption that the volumes of wine supplied and those in the culture medium were additive, so

$$V = V_0 + q_0 * t$$
$$X = X_0 + q_0 * C_i * t$$
$$E_{\text{estimated}} = \frac{X}{V} = \frac{X_0 + q_0 * C_i * t}{V_0 + q_0 * t} * 100$$

where V [L] is the volume of the fermentation medium at a given time during the loading process, V_0 [L] the volume at the start of the loading process, q_0 [L/h] the wine feed flow-rate, C_i [L/L] the ethanol concentration in the wine feed, $E_{\text{estimated}}$ [°GL] that in the culture medium at a given time, X [L] the volume of ethanol in the fermentation tank at such a time and X_0 the volume of ethanol in the tank at the start of the loading process.

The volumes of the wine feed and culture medium were previously confirmed to be additive.

Bearing in mind that this is a cyclical process in which fermentation did not stop at any time, and that the first stage is preceded by another in which acetification rate is very high, it should not be assumed that ethanol uptake rate for acetic acid production be null during the first stage. However, taking into account the dilution effect on the bacteria concentration as well as the sudden changes in the broth properties because the wine addition, it could be expected a not very important ethanol consumption by bacteria. Additionally, since this step takes a small percentage of the overall cycle time, directly it might be eliminated for the calculation of the overall reaction rate.

During the second step, where the ethanol concentration is kept constant, the fermentation rate can be estimated from the temporal variation of the volume (see the staircase curve of Fig. 3). In order to estimate the fermentation rate, one can fit the experimental data to the following function:

$$V = a * e^{b*t}$$

where a and b are constants (see Fig. 3).

Therefore, the temporal variation of the volume will be directly proportional to the rate of ethanol uptake by bacteria:

$$\frac{dV}{dt} = b * a * e^{b*t} = b * V,$$
$$\frac{1}{V}\frac{dV}{dt} = b.$$

Because the specific rate of volume change is constant, so will the biological oxidation rate. The latter can be estimated from an balance of ethanol in the medium. The rate of ethanol uptake by transformation into acetic acid will coincide with the difference between the amount of ethanol added per litre of culture medium per hour and the increase in the amount of ethanol present in the reactor per litre of medium per hour. The amount of ethanol added per litre of medium per hour will be

$$\frac{1}{V}\frac{dV}{dt} * E_e.$$

E_e being the ethanol concentration in the wine feed (120 mL/L). Also, the increase in the amount of ethanol in the reactor per litre of culture medium per hour will be

$$\frac{1}{V}\frac{dV}{dt} * E_m.$$

E_m being the ethanol concentration in the medium, which, as noted earlier, was kept constant at 5% (50 mL/L). Therefore, the rate of ethanol uptake will be given by

$$(-r_E) = \frac{1}{V}\frac{dV}{dt} * E_e - \frac{1}{V}\frac{dV}{dt} * E_m$$

$$(-r_E) = (E_e - E_m) * \frac{1}{V}\frac{dV}{dt} = (E_e - E_m)b$$

$$[-r_E] = \frac{\text{ethanol uptake, mL}}{L * h}$$

$$\frac{^\circ\text{GL}}{\text{h}} = (-r_E) * \frac{1}{10}$$

1/10 being the conversion factor required to express the reaction rate in °GL/h.

In our case, the rate of ethanol uptake during this step will be

$$(-r_E) = (120 - 50) * 0.0209 * \frac{1}{10} = 0.146\frac{^\circ\text{GL}}{\text{h}}.$$

Finally, the oxidation rate in the last step (viz. between the end of the loading operation and immediately prior to unloading) can be directly determined from the slope a plot of the ethanol concentration against time. The rate thus obtained from Fig. 4 was 0.22 °GL/h.

The previous data can be used to estimate the mean overall rate of ethanol uptake during a cycle. This entails obtaining a weighted average as a function of the proportion of time taken by each step in the overall cycle. Thus, if the first stage is not considered, and second and third step are assumed to take 45.3% and 50.2%, respectively, of the overall time (see Fig. 1), the mean overall rate of ethanol uptake will be

$$(-r_E)_{\text{global}} = 0.453 * (-r_E)_{\text{second step}} + 0.502 * (-r_E)_{\text{third step}}$$

which, upon substitution of the ethanol uptake rates, yields

$$(-r_E)_{\text{global}} = 0.453 * 0.146 + 0.502 * 0.224 = 0.179\frac{^\circ\text{GL}}{\text{h}}.$$

Besides, the mean reaction rate can also be calculated from the final acidity at the time the reactor is unloaded. Because such an acidity was 10.5 °Acetic acid, the unloaded volume 4 L, the cycle time 32.6 h and the mean overall fermentation volume 7.35 L, the reaction rate was

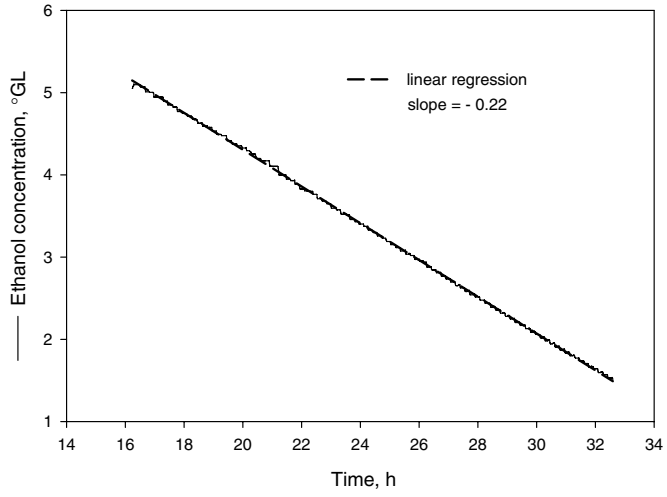

Fig. 4. Variation of experimental and fitted ethanol concentration during the final step of the cycle.

$$(r_A) = \frac{\text{final acetic acid concentration}\left(\frac{\text{g acetic acid}}{\text{L medium}}\right) * \text{unloaded volume(L medium)}}{\text{cycle time(h)} * \text{mean overall volume(L)}}$$

$$(r_A) = \frac{\frac{10.5\text{ g acetic acid}}{0.1\text{ L medium}} * 4\text{ L medium}}{32.6\text{ h} * 7.35\text{ L}} = 1.75\frac{\text{g acetic acid}}{\text{Lh}}$$

$$\Rightarrow 0.175\frac{^\circ\text{acetic acid}}{\text{h}}$$

Taking into account that 1 °GL is approximately equal to 1 °Acetic acid, the (r_A) value differs by only 3% from $(-r_E)_{\text{global}}$.

4. Conclusions

The proposed method allows the simple, fast estimation of the mean acetification rate during a typical semi-continuous vinegar production cycle. To this end, it uses the variation of the ethanol concentration during a cycle, which can be readily determined on-line and allows the mean biological oxidation rate in each step of the process to be conveniently estimated.

These data allow one to accurately quantify bacterial activity during an acetification cycle and the way it is influenced by the operating conditions with a view to identifying the specific steps most markedly affected by changes in such conditions.

In the absence of ethanol entrainment losses, the mean overall acetification rate should coincide, when other minor ethanol uses are negligible, with that estimated from the final acidity of the culture medium immediately prior to unloading the reactor.

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