PILOT SCALE VINIFICATIONS (100 L).
I THE CONTROLLED FERMENTATIONS FACILITY AT THE INRA IN PECH ROUGE.

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Introduction

Oenological experiments face numerous constraints.
In the winery, they are intricate because of the grape harvest seasonability (one or two months per year) but also the very difficult implementation of thorough experiments: (i) this would require working with large volumes thus complicating the realization of proper controls or duplicates, (ii) lack of personnel to assist experiments during this period…
Conversely, small scale experiments allow to better control the working conditions and raw materials would be readily available for longer periods, but the applicability of results obtained can be questionable, especially with regards to the characteristics of the wines produced.
Therefore, pilot scale studies would appear to be a good compromise, whose oenological advantages, however, have not been well discussed. We found it opportune to contribute to this area, specifically because we have a pilot facility at our disposal since 2001 allowing (i) the sterile storage of musts (up to 210 hl) for a year, (ii) to ferment these musts in a series of tanks equipped to precisely follow and control fermentations thanks to the in-line measurement of current fermentation rates, and (iii) allowing post-fermentation treatments as well as the physiochemical and organoleptic analysis of wines.
In this first article, we describe this facility as an example of the potential of experimental settings at pilot scale.

Description of the fermentation facility

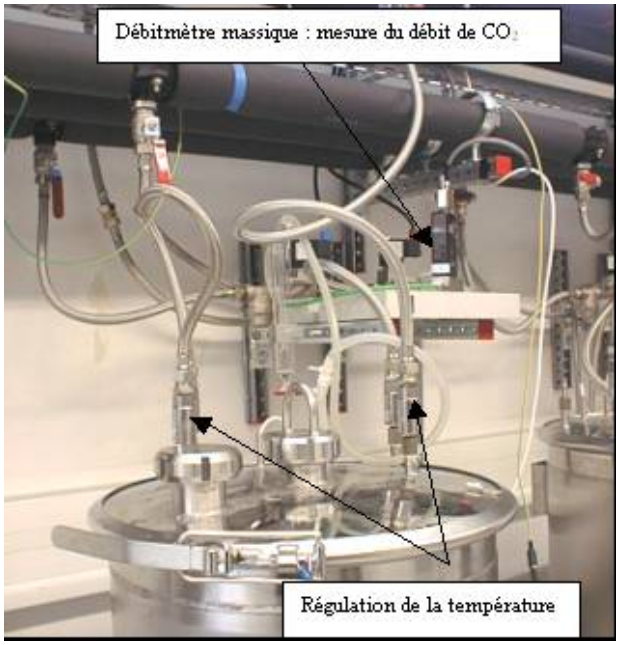
➢ Tanks

16 fermentation units are available (partial view in picture 1). The 100 l stainless steel tanks (316L) are 400 mm in diameter and have a usable height of 800 mm. The removable head plate (picture 2) is fitted with several ports, one of which is used for sample taking (51 mm diameter tube immersed in the must and thus allowing sample taking without degassing the tank) and one for CO_2 discharge. There is also a central fitting used for cap punching during red vinifications, as well as two temperature control coils, and a temperature probe allowing to measure the temperature in the middle of the tank.

➢ Temperature control:

Temperature regulation in the tanks is carried out by circulating water through two coils (length of each coil: 95 cm, diameter: 12 mm), i.e. opening or closing electro-valves, which control a hot water circuit (temperature around 40°C) fed by a warm water tank, and a cold water circuit (water temperature between 10 and 15°C according to desired temperature profile) (picture 2). Both systems have been designed in a way (Tickelman design) to achieve the same flow through all coils regardless of the number of tanks in use.
The controls have two settings (fully opened/closed) with a dead band allowing temperature regulation within 0.1°C.

Picture 1: fermentation facility



Picture 2: detail of tank head plate

- **Oxygenation control**

Oxygen is supplied by a diffuser placed in a must recirculation coil. A calibration was performed initially in order to assess the amount of oxygen that is effectively transferred into the medium (Gerland et al., 1998). This calibration allowed to calculate the oxygen transfer coefficient (kl_a), and thus, the oxygen transfer rate. Since this coefficient remains at an almost constant value during fermentation (Blateyron et al., 1998), it is possible to assess the quantity of oxygen transferred to the yeast for specific operation conditions (type of diffuser, oxygenation rate, recirculation flow…) regardless of the fermentation phase. Generally, we use an Air-Liquide diffuser (N10A) with an oxygenation rate of 5 l/h and a recirculation flow rate of 20 hl/h, which corresponds to a 2.27 h^{-1} Kl_a value and an oxygen transfer rate of 1.2 mg l^{-1} min^{-1}. Thus, a 5 minute oxygenation allows to add 6 mg of oxygen per litre. It should be mentioned that (i) this moderated addition rate and (ii) the simultaneous oxygen consumption by the yeast, allow to strongly limit, or even completely avoid the risks of must oxydation.

- **In-line measurement of CO_2 production**

- Principle

Fermentation monitoring is carried out by the automatic measurement of the CO_2 production. Indeed, there is a direct correlation between CO_2 production, sugar degradation and alcohol production (El Haloui et al. 1988). Integration of this value allows to calculate the quantity of CO_2 produced and thus, to estimate sugar residues and alcohol concentrations.

- Technology

The massic flowmeter selected to measure the production of CO_2 (Brooks TR), works as follows: gas produced from fermentation escapes through a shunt with a very small inner diameter. The proportion of gas flowing through each side of the shunt is constant. The separator wall in the shunt is homogeneously heated with an electrical resistance. Two sensors measure the temperature gradient in the gas flow. The gas flow rate is proportional to this gradient. For this, the gas has to be sufficiently dry, which requires a water trap and a condenser.

The maximum flow of the meters used at this scale is 140 l/h.

Fermentation procedures

➢ System monitoring/control

The fermentation control system has been specifically developed by INRA. It is a flexible system (e.g. new sensors can be added) controlled by a computer application programmed in Labview, which allows to:

- monitor fermentations: the CO_2 production rate is measured every 20 seconds (frequency is adjustable). The values are averaged for the duration between two data storage points (generally 20 minutes apart). The temperature is measured with a pt100 probe at 5 sec. acquisition intervals (frequency is adjustable).
- store parameters: time, current CO_2 production rate (g/l.h), total CO_2 production (g/l), set temperature (°C), measured temperature (°C) and activity of hot and cold water valves.
- control fermentations: several temperature control regimes are possible (see following section)

➢ Examples of temperature control regimes

The following examples describe different set-ups with regards to temperature control, as well as the advantage of in-line monitoring of the fermentation rate, which is much more precise, and thus more informative, than manual monitoring (e.g. by density measurement).

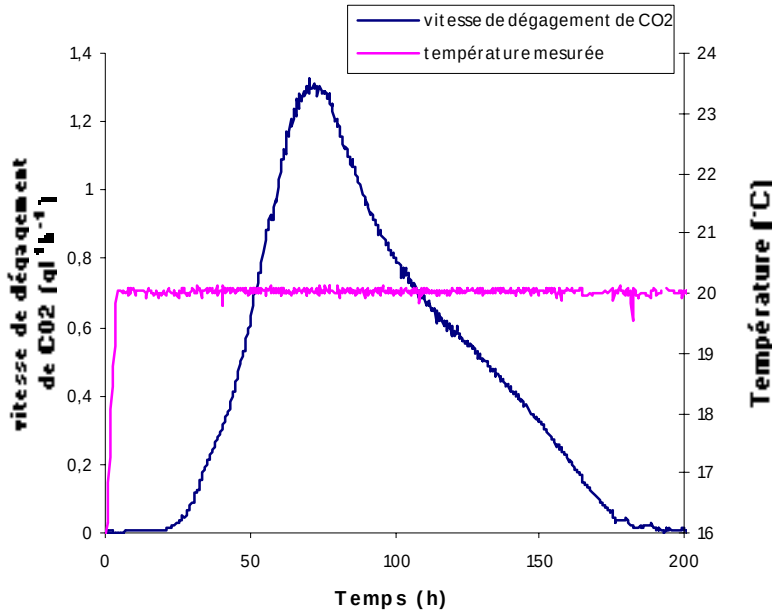


Figure 1

- Isothermal regulation (figure 1)

This is the most simple control method: The CO_2 production rate reaches a maximum and, as soon as cell growth is completed (shortly after the maximum CO_2 production rate was reached), gradually falls until the end of the fermentation. As exemplified in Figure 1, this decrease is very strong at the end of fermentation as soon as residual sugars become limiting. In the case of sluggish fermentations (examples in Figure 4 and 5), this decrease is much more progressive.

CO_2 production rate
measured temperature
X: Time [h]
Y2: Temperature [°C]
Y1: CO_2 production rate [g l^{-1} h^{-1}]

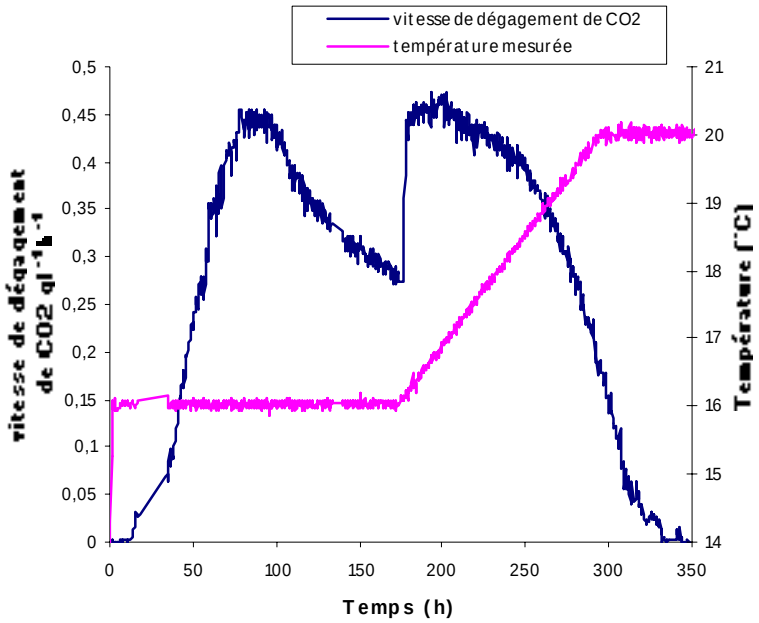


Figure 2

- Temperature regulation according to fermentation time (Figure 2)
The temperature can be regulated according to the fermentation time. As shown in Figure 2, keeping the temperature at 16°C at the beginning of the fermentation allowed to limit the maximum fermentation rate (which is generally desired). The temperature was then progressively increased to allow a faster completion of the fermentation. Please note that in this case, 300 mg/l of di-ammonium phosphate were added (at t = 150 h) whose effect (immediate and very significant) could be quantified precisely.

CO_2 production rate
set temperature
measured temperature
X: CO_2 produced [g l^{-1}]
Y2: Temperature [°C]
Y1: CO_2 production rate [g l^{-1} h^{-1}]

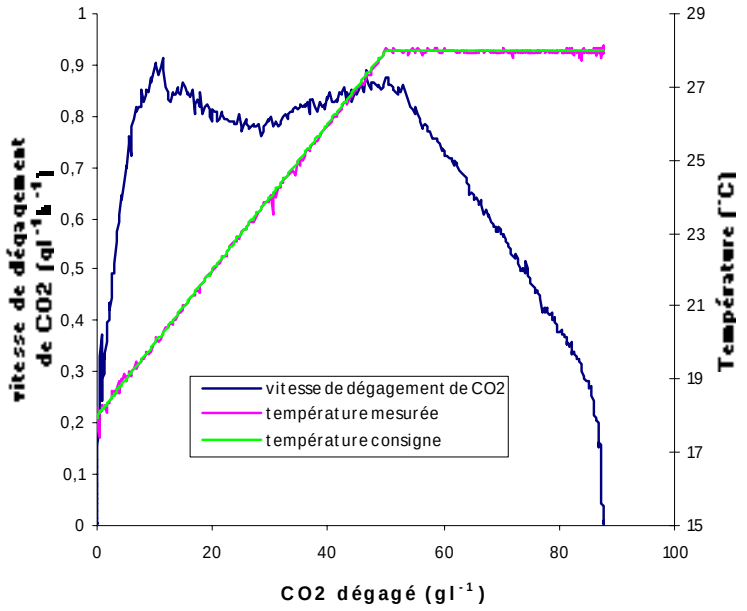


Figure 3

-Temperature regulation according to CO_2 production (figure 3)
The temperature can also be regulated according to the CO_2 production, which is proportional to the evolution of the fermentation, as well as to the sugar degradation and alcohol production. Compared with the previous regulation method, this one allows independence from the time factor while considering the medium composition. This regulation method allows to integrate one of the main characteristics of musts: the variability of their fermentability depending on their nutritional composition. Please note the congruence of the set and measured temperatures.

CO_2 production rate
measured temperature
X: Time [h]
Y2: Temperature [°C]
Y1: CO_2 production rate [g l^{-1} h^{-1}]

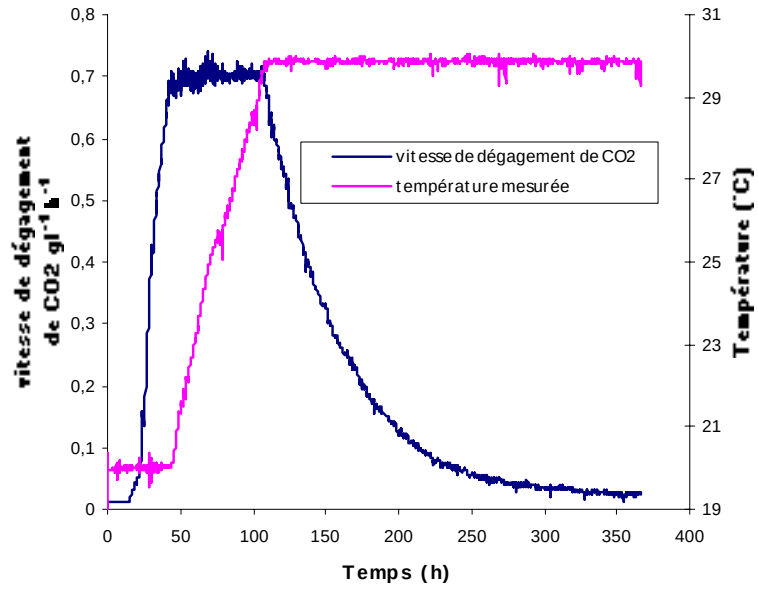


Figure 4

- Control of the fermentation rate through temperature regulation (Figure 4)
During most of the fermentation time, the fermentation rate diminishes because of decreasing yeast activity (inhibitions and nutrient limitations). This decrease can be compensated by a controlled temperature increase. This allows fermentations (or fermentation stages) where yeast activity is constant. This regulation technique is particularly interesting for studies concerned with the control of yeast fermentation activities. In figure 4, the rate is kept constant at its maximum value (at 20°C) until reaching the maximum temperature (in this case, 30°C).

- Simulation of the temperature profile in industrial tanks (Figure 5)
Under practical conditions, i.e. in industrial size tanks, there are long periods during which the temperature profile is non-isothermal, and the temperature rises freely (until the final set-point is reached). If these temperature courses are to be reproduced in 100 l tanks, which have very different temperature losses, it becomes necessary to use a simulation module as part of the control software. On the one hand, this module allows to calculate the heat production rate (proportional to fermentation rate), and on the other hand, the thermal losses. Thus, it is possible to reproduce a temperature profile similar to the ones of industrial size tanks.

CO_2 production rate
measured temperature
set temperature
X: Time [h]
Y2: Temperature [°C]
Y1: CO_2 production rate [g l^{-1} h^{-1}]

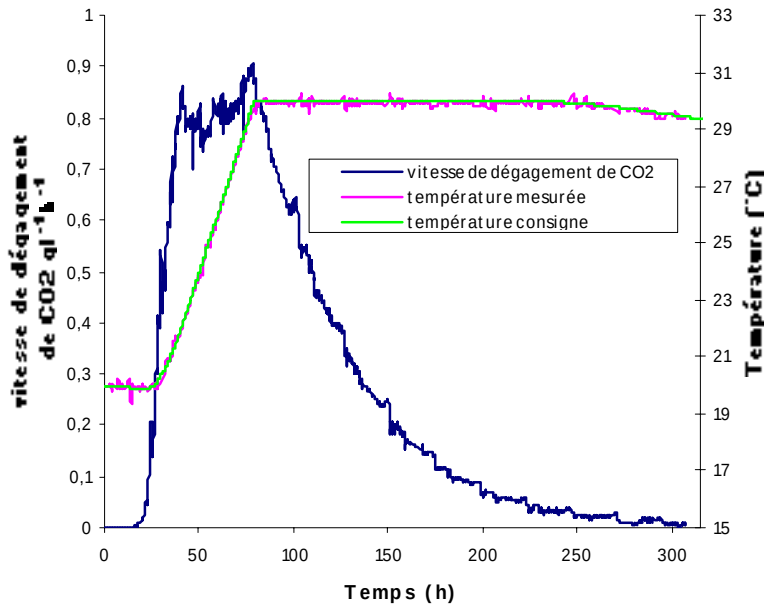


Figure 5

Fermentation repeatability

- **White wine vinifications**

Figure 6 shows the fermentation kinetics obtained with two different strains during duplicate fermentations. The duplicates are completely congruent including the phases of nutrient

addition and the repeated temperature increases (these actions were carried out at the same time point for both strains). Thus, this facility enables to demonstrate small but significant differences between experimental conditions (in this case, the yeast strain effect).

Strain A, 1st replicate
Strain B, 1st replicate
Strain A, 2nd replicate
Strain B, 2nd replicate

X: Time [h]
Y1: CO_2 production rate [g l^{-1} h^{-1}]

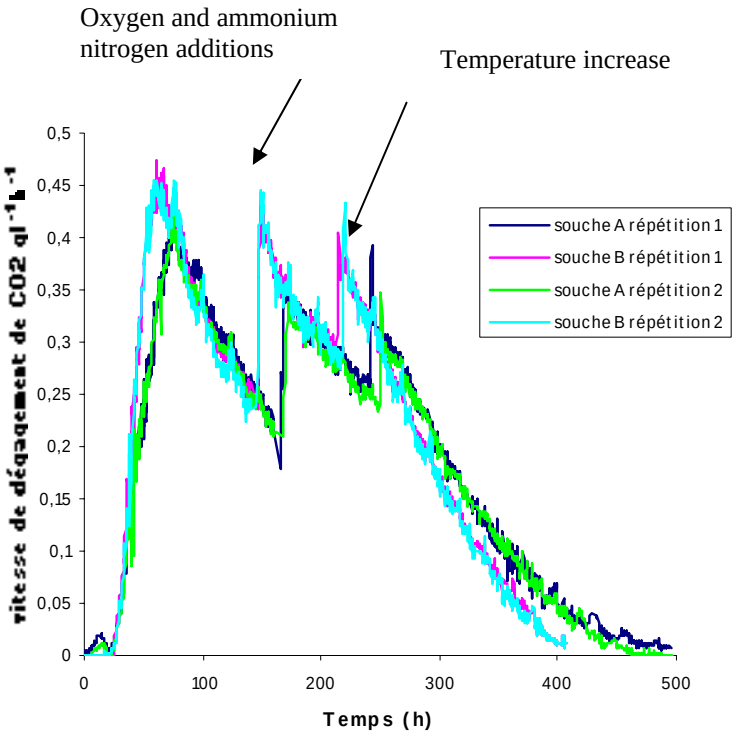


Figure 6. Comparison of the fermentation kinetics of 2 yeast strains (duplicate fermentationst)

- **Red wine vinifications**

- Repeatability

In order to obtain very good repeatabilities (see Figure 7), also including daily cap-punching, the different batches for red wine vinifications have to be homogeneous. This requires manual grape harvesting.

X: Time [h]
Y1: CO_2 production rate [g l^{-1} h^{-1}]

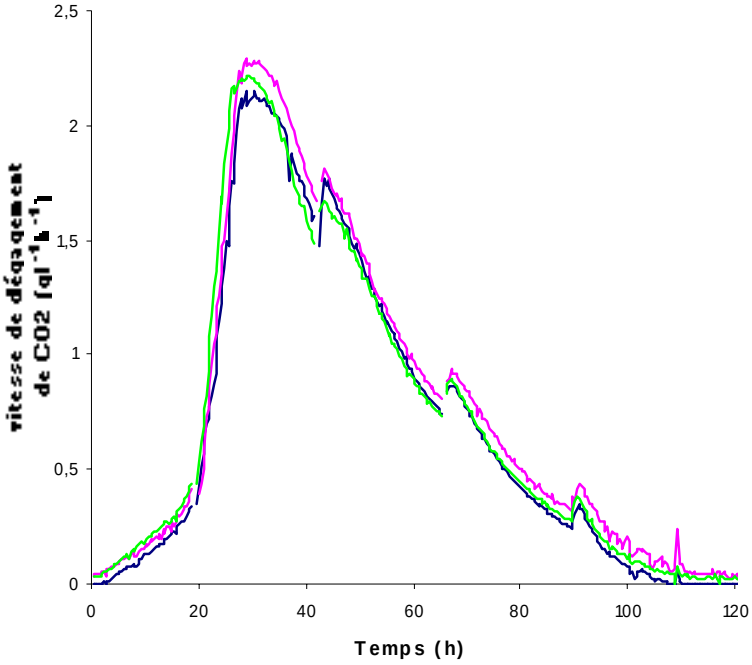


Figure 7. Repeatability of fermentation kinetics for red wine vinifications (with one daily cap-punching)

Conclusions

A facility such as the Experimental Unit in Pech Rouge, allows to implement pilot scale fermentations in a perfectly controlled and reproducible way. Moreover, the monitoring can be very precise and specific protocols can be implemented, which are specifically and highly interesting for the study of fermentations under non-isothermal conditions. In the next articles, we will present the experiments carried out at this scale (rationale, applicability…) for white and red vinifications alike (part II). Then, we will describe the effect of must storage (for several months) on the fermentation course and wine characteristics (part III).

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