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IMPEDANCE SPECTROMETRY FOR MONITORING ALCOHOLIC FERMENTATION KINETICS UNDER WINE-MAKING INDUSTRIAL CONDITIONS

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Abstract –. This paper presents a system for monitoring several parameters during alcoholic fermentation in wine industry. Alcoholic fermentation of grape juice is a quite complex biological process that involves a large number of variables and boundary conditions. The number of cells and total production of carbon dioxide are two important parameters for monitoring this process and several decisions must be carried out depending on these values. This paper presents an on-line application of impedance spectrometry to estimate both, CO_2 and cells in a small-scale fermentation plant. Experimental results has been obtained from several micro-fermentation, verifying the capability of carbon dioxide measurement and the estimation of yeast cell concentration.

Keywords: impedance spectrometry, fermentation, wine

1. INTRODUCTION

Wine making from grape juice to finished wine is a complex process that involves several stages, often conducted empirically and traditionally. Most important steps are the skin maceration and the alcoholic fermentation that determines final organoleptic characters. Alcohol production during fermentation process can be determined by measurement of total carbon dioxide release [1], [4]. So, fermentation kinetics could be controlled (or followed) by measurement of total carbon dioxide.

Carbon dioxide and alcohol production is a consequence of biological activity of cells during fermentation stage. Thus, to improve control actions, total number of cells should be obtained in addition to carbon dioxide production. Variables, CO_2 and total cell count should be on-line measured in order to provide criteria for process controlling.

On-line measurement of carbon dioxide in liquid (grape juice to wine) can be carried out by means of resistance, due to effect of CO_2 bubbles (carbon dioxide bubbles increase the value of total resistance). Cell count affects to real permittivity [2], [3] and can be obtained by capacitance measurement at appropriate frequency.

This paper describes a system for monitoring both, cell count and CO_2, by using impedance spectrometry; in next sections, a brief system description and several experimental results will be presented.

2. MEASUREMENT SYSTEM

Measurement system has been included into a full-controlled micro-fermentation plant as it is shown in Fig. 1 able to deliver 1000 to 2000 ml of grape juice. It consists in a vessel that contains the grape juice and an impedance cell for liquids from Hewlett-Packard (HP16452A). Liquid circulates from bottom of vessel to impedance cell and from this one to the vessel by means a controlled peristaltic pump to avoid any change in bubbles production.

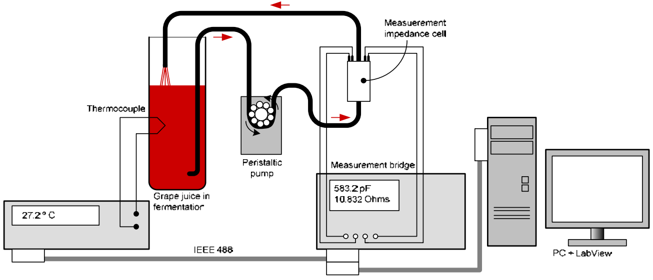

Fig. 1. Micro-fermentation plant including on-line measurement of carbon dioxide and cell count by means of impedance spectrometry.

Capacity and resistance values are calculated in a measurement bridge (impedance analyzer from Hewlett-Packard (HP4284A) and liquid temperature is also measurement by means of a K-type thermocouple to compensate its effect on resistance measurement. All measured values are transmitted via IEEE-488 to a standard PC that includes a LabView program able to store, display and manage all data.

Fig. 2 shows the impedance measurement cell and its electrical equivalent circuit when grape juice is the liquid inside: a parallel circuit with a capacitance, C_P and a resistance, R_P. But, grape juice has a lot of dissolved ions and consequently, it has a high value of conductivity. So, R_P produces a low value – around 10 Ohms for the particular geometry of impedance cell, characterized by a distance between electrodes of 2.6 mm – introducing additional problems in capacitance estimation because R_P seems a short-circuit. An increase of electrodes distance of measurement cell will increase the value of R_P but it would reduce of measured capacity C_P.

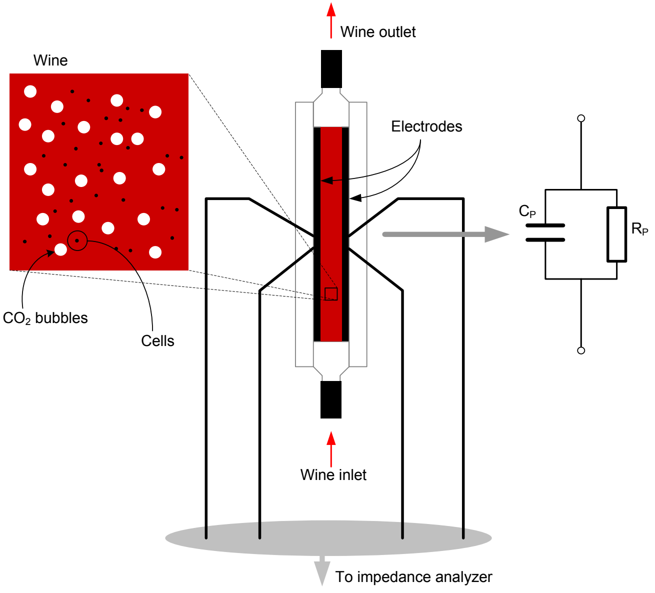

Fig. 2. Impedance measurement cell with grape juice (or wine) inside connected to impedance analyzer modelled with a resistive-capacitive equivalent circuit. Carbon dioxide and cells in liquid affects to equivalent circuit parameters.

The effect of carbon dioxide can be well modelled by liquid conductivity, that is, carbon dioxide bubbles reduce the effective section of liquid conductor in impedance cell, increasing total resistance. Additional effects, such as changes in intrinsic conductivity due to liquid composition modification become negligible in relation to large effect of bubbles. Conductivity in liquid is measurement with AC excitation to avoid polarization effects caused by dissolved ions,

The effect of any kind of cell over real permittivity of liquid and, consequently, over capacity value, has been well established for other high-conductivity liquid such as raw milk with presence of somatic cells [2], [3]; the quantitative effect over capacity is depending on cell size and excitation frequency.

2.1. Impedance spectrometry during wine-making

The micro-fermentation workbench in Fig. 1 can be used for any kind of impedance spectrometry of liquids, but must be adapted to the particular conditions of wine-making.

Under the special conditions of grape juice fermentation, and when *Saccharomyces Cerevisiae* is used as yeast, excitation frequency must range from 300 kHz to 1 MHz for capacity measurement and around of 40 kHz for resistance (or conductivity) measurement. However, no significant differences were found in resistance measurement when excitation frequency ranges from 4 to 400 kHz.

2.2. Thermal effects

Temperature is a controlled variable in industrial wine-making process because it affects to the fermentation kinetics and other processes. In our laboratory-level plant for micro-fermentation, temperature was not controlled and its evolution depended on room conditions; it needs a minimum value to start and to hold the fermentation and this minimum was guaranteed.

However, monitoring system of Fig. 1 reads the temperature of grape juice, because the conductivity – and electrical resistance – of liquids has a heavy thermal-dependence. There are models for thermal dependence of conductivity of pure water and ultra-pure water (high resistance liquids) but these models are no valid for high-conductivity liquids such as wine and grape-juice. For this case we will use a linear model because temperature range is enough short to consider non-linearity effect as negligible.

$$R_T = R_{TREF}\left(1+\alpha\left(T-T_{REF}\right)\right) \qquad (1)$$

where R_T is the measured resistance at the measured temperature T, R_{TREF} is the resistance at T_{REF}, and α is the thermal coefficient.

3. EXPERIMENTAL RESULTS

Several micro-fermentation processes have been carried out by using the system shown in Fig. 1 with 1500 ml of “tempranillo” grape juice with 0.8 g of *Saccharomyces Cerevisae* and 1 g of fermentation activator. In all fermentation processes, total time involved was 10 days (248 hours), time enough to complete fermentation [5], [6] under the specific conditions of this experiment. The evolution of variables is stored by means a custom LabView program with 30 min of sample period. In all cases, sampled variables are: liquid temperature, electrical capacity, C_P and parallel resistance, R_P of equivalent electrical model shown in Fig. 2 for liquid impedance cell. These values are measured from 100 Hz to 1 MHz with a logarithmic sweep.

All measured variables exhibit similar behaviour in all fermentation processes carried out; for example, Fig. 3 shows the value of measured resistance, R_P at 40 kHz of excitation frequency– found values of R_P are quite similar from 400 Hz to 400 kHz – and Fig. 4, the liquid temperature during a complete fermentation.

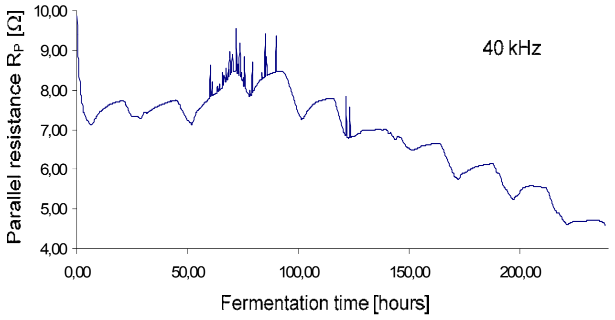

Fig. 3. Evolution of liquid resistance, R_P at 40 kHz during fermentation stage of wine-making (240 hours).

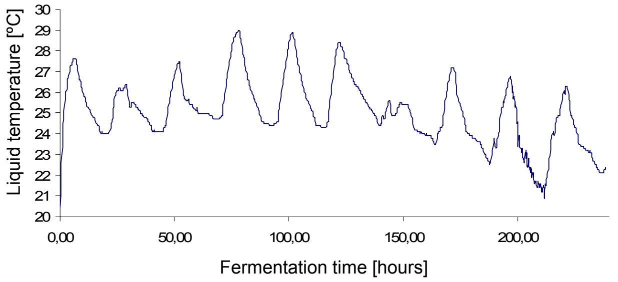

Fig. 4. Liquid temperature during fermentation stage (240 hours).

Thermal effects on measured resistance of high-conductivity liquids can be compensated by means an expression such as (1). In the case of grape juice, thermal coefficient α in that equation can be calculated from the micro-fermentation workbench of Fig. 1, using values where changes are due to temperature because liquid has not biological evolution: Fig. 5 shows the relationships between temperature and resistance in that case and we can verified a high correlation when the thermal model of (1) is used.

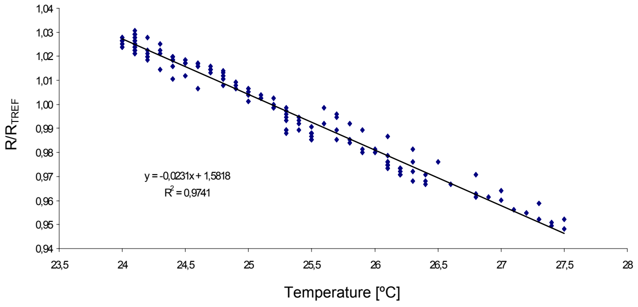

Fig.5. Relationship between temperature of liquid a normalized resistance R/R_{TREF}. A high correlation coefficient was found for a model similar to (1).

The obtained thermal model from Fig.5 can be presented as:

$$R_T = R_{25^\circ C}\left(1 - 0.0231\left(T - 25^\circ C\right)\right) \qquad (2)$$

where R_T is the measured resistance at temperature T. Similar empirical procedure applied to other types of grape juice has produced similar values of the thermal coefficient α, so, it could be considered as a constant for grape juice.

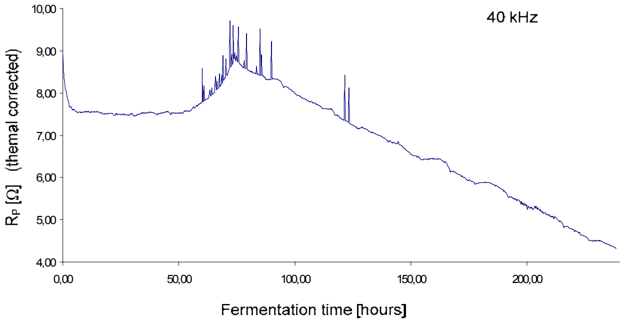

Fig. 6. Evolution of liquid resistance, RP at 40 kHz (thermal correction is applied) during fermentation stage of wine-making (240 hours).

Values of measured parallel resistance of Fig. 3 has been corrected by means of expression (2), producing the curve of Fig. 6.

Parallel resistance value, R_P after temperature correction exhibits similar behaviour to carbon dioxide production in alcoholic fermentation process [5], [6], presenting a maximum value when the fermentation produce the maximum carbon dioxide release. This effect is also verified by notable visual effects such as a huge production of bubbles in liquid and tumultuous fermentation in grape vessel.

The value of capacity C_P during fermentation presents dependence on frequency as it can be seen in Fig. 7 but all of cases exhibits similar evolution including some influence of liquid temperature.

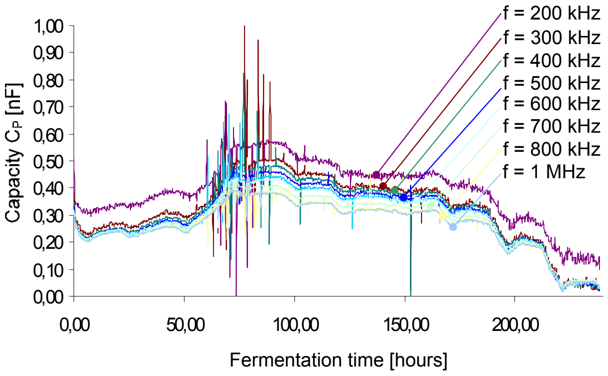

Fig. 7. Evolution of capacity, C_P during fermentation stage of wine-making (240 hours) in function of excitation frequency. Speaks in measured value are due to perturbation caused by presence of huge CO_2 bubbles, and could be removed by a simple averaging.

The evolution of measured capacity, C_P – at any frequency from 200 kHz to 1 MHz – is similar to the evolution of yeast cell concentration [5], [6]: after 50 hours of beginning, the number of yeast cells increase up to its maximum concentration during tumultuous fermentation and then, it decreases slowly to the end of fermentation. The evolution of measured resistance, R_P at any excitation frequency completes the information about fermentation kinetics but, in this case, is focused in carbon dioxide release. Both values, capacity C_P and resistance R_P are plotted in Fig. 8 to show how they can explain the fermentation kinetics:

- First stage: from process beginning, the total count of yeast cells increase slowly and there is not visible carbon dioxide bubbles. This stage is characterized by a constant value of liquid resistance and a slow increase of capacity.
- Fermentation starts with a multiplication of yeast cells and they begin to transform sugar in alcohol, causing an increase of CO_2 release. This instant is defined by a hard increase in slope of R_P and C_P.
- Tumultuous stage: there are a lot of yeast cells and they are extremely active, causing the maximum release in carbon dioxide. R_P reaches it maximum value and C_P keeps in high values.

- After the tumultuous stage, fermentation continues, but the CO_2 release decrease due to the consumption of sugar and the cell count begins to fall. These effects are represented, respectively by the decrease of C_P and R_P.
- End of fermentation, without gas release because most of sugar has been yet transformed in alcohol. C_P and R_P have their minimum values.

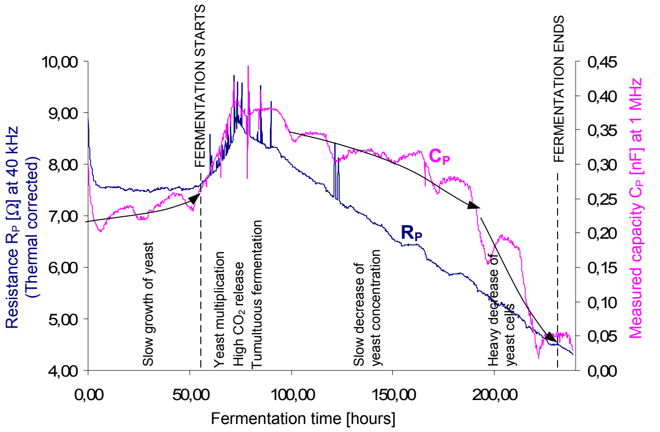

Fig. 8. Evolution of capacity, C_P during fermentation stage of wine-making (240 hours) in function of excitation frequency. Speaks in measured value are due to perturbation caused by presence of huge CO_2 bubbles, and could be removed by a simple averaging.

4. A NEW CARBON DIOXIDE SENSOR BASED UPON IMPEDANCE SENSOR

A new carbon dioxide sensor has been designed according to the results obtained from workbench of Fig.1 during micro-fermentation. This sensor consists of two plates of stainless steel 304 sunken in liquid, those impedance is measured by means an AC Wheatstone bridge excited at 40 kHz with thermal correction following (2). The equivalent model is not a pure-resistive circuit and some capacitive effect must appear, but the distance between both plates (electrodes) is enough to reduce that disturbance. Thus, impedance measured will be similar to liquid resistance between two plates.

This sensor and the conditioning circuit have been used under industrial condition fermentation and their results are compared to the values produced by a commercial CO_2 sensor. Fig. 9 shows this comparison.

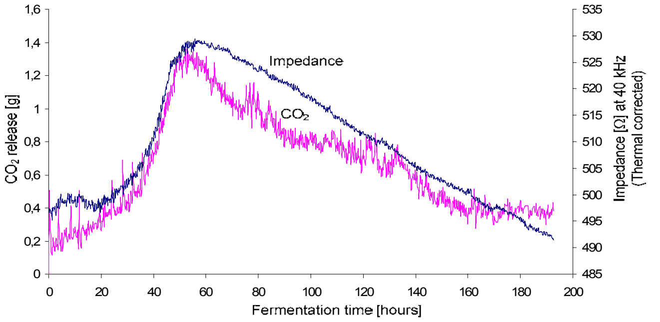

Fig. 9. Comparison of carbon dioxide release and measured impedance in an industrial fermentation.

As we can see, the values of impedance sensor exhibits a similar evolution that values provided by a commercial CO_2 sensor, with perfect agreement in determination of fermentation start time and the instant of maximum gas release. Small differences were found after maximum gas release point, due to the additional capacitive effect of yeast cells.

Fig. 10 plots the value of gas release in function on measured impedance and we can found a high correlation that can validate this kind of sensor.

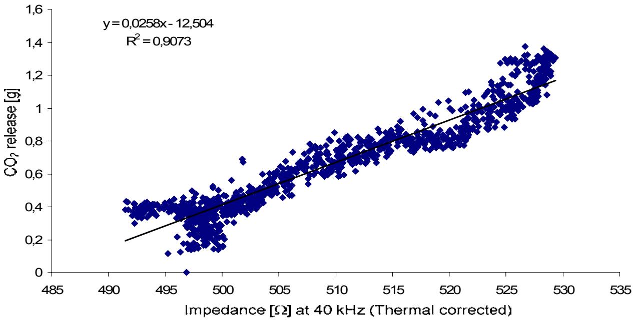

Fig. 10. Use of liquid impedance to model CO_2 release.

But, if we verify the time evolution of two variables – carbon dioxide release and resistance – we can see that both parameters have a similar behaviour except for a small area after the maximum value of gas release, when the concentration of yeast cells reaches its maximum value, as we can see in Fig. 10.

The interest of the estimation of CO_2 is due to the relationship between the evolution of must mass that controls the moment of fermentation and the carbon dioxide release as we can see in the Fig. 11.

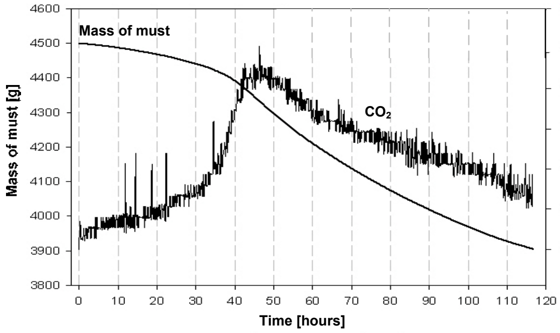

Fig. 11. Comparison of carbon dioxide release and the must quantity to demonstrate why the gas release is a very interesting variable to predict the kinetic of fermentation process.

5. CONCLUSIONS

Analysis of resistance and capacitance can be used to estimate the kinetics of alcoholic fermentation during wine-making process. After the results obtained from a micro-fermentation plant, measured resistance after temperature compensation can be used to estimate carbon dioxide release, and the total number of yeast cells could be estimated with capacitance at high frequencies (0.2 to 1 MHz). Finally, a CO_2 sensor based upon liquid impedance has been designed as a consequence of experimental results. This sensor has been verified during real fermentation process under industrial conditions and provide similar results that very expensive commercial CO_2 sensor.

ACKNOWLEDGMENT

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