A model for pH determination during alcoholic fermentation of a grape must by *Saccharomyces cerevisiae*

Huberson Akin, Cédric Brandam, Xuân-Mi Meyer*, Pierre Strehaiano

Laboratoire de Génie Chimique, UMR CNRS/UPS/INPT 5503, BP 1301, 5 rue Paulin Talabot, 31106 Toulouse Cedex 1, France

Abstract

A model to predict accurately pH evolution during alcoholic fermentation of must by *Saccharomyces cerevisiae* is proposed for the first time. The objective at least is to determine if the pH measurement could be used for predictive control. The inputs of the model are: the temperature, the concentrations in sugars, ethanol, nitrogen compounds, mineral elements (magnesium, calcium, potassium and sodium) and main organic acids (malic acid, citric acid, acetic acid, lactic acid, succinic acid). In order to avoid uncertainties coming from the possible precipitation, we studied this opportunity on a grape must without any tartaric acid, known as forming complexes with potassium and calcium during the fermentation. The model is based on thermodynamic equilibrium of electrolytic compounds in solution. The dissociation constants depend on the temperature and the alcoholic degree of the solution. The average activity coefficients are estimated by the Debbye–Hückel relation. A fictive diacid is introduced in the model to represent the unmeasured residual species. The molality of hydrogen ions and thus the pH are determined by solving a non-linear algebraic equations system consisted of mass balances, chemical equilibrium equations and electroneutrality principle. Simulation results showed a good capacity of the model to represent the pH evolution during fermentation.

Keywords: Fermentation; pH; Model; *Saccharomyces cerevisiae*; Grape must

1. Introduction

pH is currently used in oenology as an indicator of different aspects: contamination risks, efficiency of sulphating, sensorial properties. Contaminations of grape musts or wines by bacteria or yeasts like *Brettanomyces* sp. are easier at high pH values and the prevention of contaminations by SO_2 addition is inefficient when the pH value is too high [1,2]. Acidity also plays an important role in sensorial analysis for wines and even if pH and acidity taste are not totally correlated, pH can give information on this organoleptic property. As it is quite easy to measure, pH could be used not only at the end of the process to qualify the end product but also as an indicator throughout the fermentation. In fact, during the alcoholic fermentation of grape musts, the conversion of substrates (sugars, organic acids, nitrogen) into metabolites such as ethanol and organic acids by the yeasts *Saccharomyces cerevisiae* modifies the thermodynamic equilibrium in the medium and consequently the pH. Moreover, pH has a great impact on the activity of cell enzymes and can modify the chemical pathways of the biological reactions as well as their kinetics.

To predict pH evolution during wine fermentation two kinds of models are necessary: one to calculate pH knowing the composition of the broth and one representing the dynamic of the fermentation to determine the evolution of the composition of the broth. Many papers deal with kinetic models for the production of biomass [3,4], or metabolites [5,6], but studies on pH modelling are more limited. The works of Ratsimba [7], Gerbaud [8] and Devatine [9] on the prediction of the crystallization of tartaric salts in hydro-alcoholic solutions are interesting but they were developed for wine like composition media. Nevertheless, the concept of vinic acid introduced by these authors to overcome the incapacity to identify all the compounds involved in a wine caught our attention and was extended in our study to predict pH of grape musts and fermentation broth.

This work aimed to better understand the influence of compounds produced or consumed during fermentation process and

* Corresponding author.
E-mail address: Xuan.Meyer@ensiacet.fr (X.-M. Meyer).

to capitalize this knowledge in a mathematical model which calculates pH value from the composition of musts. The objective at least is to determine if the pH measurement could be used as an indirect evaluation of the kinetic reactions of the fermentation and if this measurement could be used as a controlled parameter for predictive control. In order to avoid introducing uncertainties coming from the possible precipitation, we studied this opportunity on a grape must without any tartaric acid, known as forming complexes with potassium and calcium during the fermentation.

2. Materials and methods

2.1. Microorganism

The microorganism is *S. cerevisiae* QA-23 commercialised by Lallemand Company.

It is a classical strain for white winemaking.

2.2. Fermentation

This section describes the different steps and culture media to carry out the fermentation.

2.2.1. First step pre-culture

The composition of the medium was:

Yeast extract	$10\,g\,L^{-1}$
Glucose	$20\,g\,L^{-1}$
Papaïnic peptone of soya	$20\,g\,L^{-1}$

100 ml of this YPD medium were inoculated and incubated at 30 °C under agitation (150 rpm) during 24 h.

2.2.2. Second step pre-culture

The medium was composed of:

Glucose	$50\,g\,L^{-1}$
KH_2PO_4	$5\,g\,L^{-1}$
$(NH_4)_2SO_4$	$2\,g\,L^{-1}$
$MgSO_4{\cdot}7H_2O$	$0.4\,g\,L^{-1}$
Yeast extract	$1\,g\,L^{-1}$

150 ml of the medium were inoculated with 30 ml of the first step pre-culture and incubated 20 h at 30 °C under agitation (150 rpm).

2.2.3. Fermentation medium and operating conditions

The fermentation medium is a white grape must provided by SOPAGLY Company. It was concentrated up to a sugar concentration of $767\,g\,L^{-1}$ for preservation and did not contain any tartaric, acetic, succinic acid, nor ethanol. It was diluted with distilled water four times before fermentation.

This grape juice was analysed and the concentrations of the main components are given in Table 1.

Table 1
Main compounds of the white grape considered in this study

Compounds	Concentration ($g\,L^{-1}$)
Sugar	181.7 ± 4.7
Glycerol	1.62 ± 0.14
Malic acid	6.37 ± 0.09
Citric acid	1.42 ± 0.53
Potassium	0.768 ± 0.04
Calcium	0.20 ± 0.01
Magnesium	0.09 ± 0.004
Sodium	0.028 ± 0.001
Assimilable nitrogen ammonium ion (mg N/L)	74.9 ± 3.0
Alpha amino acids (mg N/L)	136 ± 5.4

The nitrogen sources necessary for the yeast metabolism were ammonia and amino acids. The must contains about $210\,mg\,L^{-1}$ of total assimilable nitrogen. No exogene nitrogen sources were added to the must.

The main mineral elements of the must are potassium, calcium, sodium and magnesium. Their contents in the grape must and during the fermentation are listed in Tables 1 and 6.

The initial cellular concentration was fixed to 8×10^6 cells mL^{-1}. Fermentation was carried out in a New Brunswick Scientific (NBS) fermentor type of 2 L at 20 °C under a 150 rpm agitation. Temperature was regulated at 20 °C. The pH was not controlled during this anaerobic fermentation.

2.3. pH and compounds measurements

Biomass concentration was estimated by two analytical methods: optical density measurement and cell numeration on a Thoma hemacytometer. A correlation between the optical density of yeast suspension at 620 nm and cell dry weight has been established. The percentage of viable cells was obtained using the methylene blue staining method during numeration, with an error of 8%.

The pH was measured using an external pH-meter (PHM210 Radiometer analytical) with a precision of 0.05 pH unit.

Concentrations of ammonium cations, alpha amino acids, malic acid and lactic acid were determined by enzymatic method (MicroDOM). The determination of the total sugar concentration was performed with the DNS method [10]. Citric acid, acetic acid, succinic acid, ethanol and glycerol were measured by HPLC method. The apparatus (TSP Spectra System) has a column (BioRad Aminex HXP-87H) specific for separation of alcohols, organic acids and sugars. The liquid phase consists of a 5 mM sulphuric acid solution circulating at $0.4\,mL\,min^{-1}$. The temperature of the column was set to 40 °C and the volume of the loop of injection was 20 μL. The detection of each component is done using a differential refractometer (TSP RefractoMonitor V).

Mineral elements (calcium ion, sodium ion, magnesium ion, potassium ion) were measured with the emission spectrometer of Horiba Jobin Yvon.

3. pH calculation model

3.1. Expression of the pH

pH expresses the activity of the hydrogen ions in a solution, using a logarithmic scale:

$$\mathrm{pH} = -\log_{10}(a_{\mathrm{H^+}}) = -\log_{10}(\gamma_{\mathrm{H^+}} m_{\mathrm{H^+}}) \tag{1}$$

3.2. Calculation of the ion activity coefficient

The activity coefficient of $H^+(\gamma_{H^+})$ was calculated with a Debbye–Hückel model with the MacInnes convention [11,21]:

$$\gamma_{\mathrm{H^+,MacInnes}} = \gamma_{\mathrm{H^+}} \frac{\gamma_{\mathrm{Cl^-}}}{\gamma_{\pm,\mathrm{KCl}}} = \frac{\gamma^2_{\pm,\mathrm{HCl}}}{\gamma_{\pm,\mathrm{KCl}}} \tag{2}$$

In wine, not to take into account this correction can lead to an error of 0.15 unit of pH [8].

3.3. Calculation of the molality of the ionic species coming from acid dissociation in solution

The usual formulation of a reactor model consists in writing a mass balance on each species in solution [12]. Then, the model includes as many variables (reaction extent) than there are reactions in the medium. The initialization of these variables is difficult and can lead to a simulation failure.

By writing the mass balance on an invariant of the reactive system, the size of the numerical problem can be reduced and the initialisation difficulty can be overcome. In the case of the dissociation of the acids, whatever their type, mono (A_IH), di ($A_{II}H_2$) or tri ($A_{III}H_3$) acid, radicals A_I, A_{II} and A_{III} are preserved. Assuming that the radicals are only involved in dissociation reactions, the following mass balances can be written:

- Mono acid (A_IH):

$$m^{\mathrm{T}}_{\mathrm{A_I}} = m_{\mathrm{A_IH}} + m_{-} \tag{3}$$

- di acid ($A_{II}H_2$):

$$m^{\mathrm{T}}_{\mathrm{A_{II}}} = m_{\mathrm{A_{II}H_2}} + m_{\mathrm{HA_{II}^-}} + m_{\mathrm{A_{II}^{2-}}} \tag{4}$$

- tri acid ($A_{III}H_2$):

$$m^{\mathrm{T}}_{\mathrm{A_{III}}} = m_{\mathrm{A_{III}H_3}} + m_{\mathrm{A_{III}H_2^-}} + m_{\mathrm{A_{III}H^{2-}}} + m_{\mathrm{A_{III}^{3-}}} \tag{5}$$

The molality of the species A_IH, A_I^-, $A_{II}H_2$, $A_{II}H^-$, A_{II}^{2-}, $A_{III}H_3$, $A_{III}H_2^-$, $A_{III}H^{2-}$, A_{III}^{3-} in solution can then be explicitly written as a function of m_{H^+}, of the activity coefficients from the previous mass balances and the chemical equilibrium constants expression.

- Mono acid (A_IH):

$$K_{1\mathrm{A_I}} = \frac{a_{\mathrm{H^+}} a_{\mathrm{A^-}}}{a_{\mathrm{AH}}} = \frac{m_{\mathrm{H^+}} m_{\mathrm{A^-}} \gamma^2_{\pm,1:1}}{m_{\mathrm{AH}} \gamma_{\mathrm{A_IH}}} \tag{6}$$

- di acid ($A_{II}H_2$)

$$K_{1\mathrm{A_{II}}} = \frac{a_{\mathrm{A_{II}H^-}} a_{\mathrm{H^+}}}{a_{\mathrm{A_{II}H_2}}} = \frac{m_{\mathrm{A_{II}H^-}} m_{\mathrm{H^+}} \gamma^2_{\pm,1:1}}{m_{\mathrm{A_{II}H_2}} \gamma_{\mathrm{A_{II}H_2}}} \tag{7}$$

$$K_{2\mathrm{A_{II}}} = \frac{a_{\mathrm{A_{II}^{2-}}} a_{\mathrm{H^+}}}{a_{\mathrm{A_{II}H^-}}} = \frac{m_{\mathrm{A_{II}^{2-}}} m_{\mathrm{H^+}} \gamma^3_{\pm,2:1}}{m_{\mathrm{A_{II}H^-}} \gamma^2_{\pm,1:1}} \tag{8}$$

- tri acid ($A_{III}H_3$):

$$K_{1\mathrm{A_{III}}} = \frac{a_{\mathrm{A_{III}H_2^-}} a_{\mathrm{H^+}}}{a_{\mathrm{AH_3}}} = \frac{m_{\mathrm{A_{III}H_2^-}} m_{\mathrm{H^+}} \gamma^2_{\pm,1:1}}{m_{\mathrm{A_{III}H_3}} \gamma_{\mathrm{A_{III}H_3}}} \tag{9}$$

$$K_{2\mathrm{A_{III}}} = \frac{a_{\mathrm{A_{III}H^{2-}}} a_{\mathrm{H^+}}}{a_{\mathrm{A_{III}H_{\acute{e}}^-}}} = \frac{m_{\mathrm{A_{III}H^{2-}}} m_{\mathrm{H^+}} \gamma^3_{\pm,2:1}}{m_{\mathrm{A_{III}H_2^-}} \gamma^2_{\pm,1:1}} \tag{10}$$

$$K_{3\mathrm{A_{III}}} = \frac{a_{\mathrm{A_{III}^{3-}}} a_{\mathrm{H^+}}}{a_{\mathrm{A_{III}H^{2-}}}} = \frac{m_{\mathrm{A_{III}^{3-}}} m_{\mathrm{H^+}} \gamma^4_{\pm,3:1}}{m_{\mathrm{A_{III}H^{2-}-}} \gamma^3_{\pm,2:1}} \tag{11}$$

3.4. Calculation of the average coefficient of activity

The calculation of the average coefficient of activity was carried out with the law of Debye–Hückel [11] (Denbigh, 1981). In the scale of the molalities, it was written as

$$\log_{10}\gamma_{\pm} = -A_{\mathrm{DH}} z_+ |z_-| \frac{\sqrt{I}}{1 + B_{\mathrm{DH}} \alpha \sqrt{I}} + C_{\mathrm{DH}} I \tag{19}$$

with

$$A_{\mathrm{DH}} = \frac{1}{4\pi \ln 10} \left(\frac{e}{\sqrt{\varepsilon k_\beta T}} \right)^3 \sqrt{\frac{\rho N_{\mathrm{A}}}{2}},$$

$$B_{\mathrm{DH}} = \sqrt{\frac{2e^2 N_{\mathrm{A}} \rho}{\varepsilon k_\beta T}} \quad \text{and} \quad C_{\mathrm{DH}} = 0.055 \tag{20}$$

α is the shortest distance of approach of the hydrated cation: 4 Å for the potassium cation and 5 Å for hydrogen cation.

The ionic force of the solution is defined by the relation

$$I = \frac{1}{2} \left[\sum_{i=1}^{n\_\mathrm{species}} m_i z_i^2 \right] \tag{21}$$

This equation can be written as

$$I - \frac{1}{2} \left[\sum_{i=1}^{n\_\mathrm{electrolytes}} m_i(m_{\mathrm{H^+}}, I) z_i^2 \right] = 0 \tag{22}$$

The electroneutrality must be ensured in the solution, so that

$$\sum_{i=1}^{n\_\mathrm{electrolytes}} m_i(m_{\mathrm{H^+}}, I) z_i = 0 \tag{23}$$

with

$$m_{\mathrm{A_I^-}} = \frac{m^{\mathrm{T}}_{\mathrm{AH}}}{1 + m_{\mathrm{H^+}} \gamma^2_{\pm,\mathrm{H:A}} / K_{1\mathrm{A_I}}} \tag{24}$$

$$m_{A_{II}H^-} = \frac{m^T_{A_{II}H_2}}{1 + \left(K_{2A_{II}}\gamma^2_{\pm,H:HA}/m_{H^+}\gamma^3_{\pm,H_2:A}\right) + \left(m_{H^+}\gamma^2_{\pm,H:HA}/K_{1A_{II}}\right)} \quad (25)$$

$$m_{A_{II}^{2-}} = \frac{m_{A_{II}H^-}K_{2A_{II}}\gamma^2_{\pm 1:1}}{m_{H^+}\gamma^3_{\pm 2:1}} \quad (26)$$

$$m_{H_2A^-} = \frac{m^T_{H_3A}}{1 + (m_{H^+}\gamma^2_{\pm 1:1}/K_{1A_{III}}) + (K_{2A_{III}}\gamma^2_{\pm 1:1}/m_{H^+}\gamma^3_{\pm 2:1}) + (K_{2A_{III}}K_{3A_{III}}\gamma^2_{\pm 1:1}/m^2_{H^+}\gamma^4_{\pm 3:1})} \quad (27)$$

$$m_{HA^{2-}} = \frac{m^T_{H_3A}}{1 + (m_{H^+}\gamma^3_{\pm 2:1}/K_{2A_{III}}\gamma^2_{\pm 1:1}) + (m^2_{H^+}\gamma^3_{\pm 2:1}/K_{2A_{III}}K_{1A_{III}}) + (K_{3A_{III}}\gamma^3_{\pm 2:1}/m_{H^+}\gamma^4_{\pm 3:1})} \quad (28)$$

$$m_{A^{3-}} = \frac{m^T_{H_3A}}{1 + (m_{H^+}\gamma^4_{\pm,H_2:HA}/K_{3A_{III}}\gamma^3_{\pm,H_2:HA}) + (m^2_{H^+}\gamma^4_{\pm,H_3:A}/m^2_{H^+}\gamma^4_{\pm,H_3:A}) + (m^3_{H^+}\gamma^4_{\pm,H_3:A}/K_{1A_{III}}K_{2A_{III}}K_{3A_{III}}\gamma_{\pm,H_3A})} \quad (29)$$

and

$$m_{OH^-} = \frac{K_w}{m_{H^+}} \quad (30)$$

The formulation of the model with explicit calculations of all the species in solutions coming from the dissociation of acid in solution enables to solve a non-linear equation systems consisting of only two equations: the ionic force equation (22), the electro-neutrality equation (23), and two unknowns: the molality in H^+ and the ionic force I.

This formulation facilitates the initialisation of the Newton–Raphson iterative procedure [13] carried out to solve the problem.

3.5. Determination of the influence of unmeasured compounds

It is impossible to know the whole composition of a real grape must and some unmeasured components may have an influence on the pH calculation. Oxalic acid, galacturonic acid, gluconic acid, glyceric acid, citramalic acid, dimethylglyceric acid are known to be present in grape must but are not measured. Anions such as SO_4^{2-} or PO_4^{3-} are neither measured. To overcome this problem the molality of a fictitious diacid, called the "vinic" acid, was introduced in the model, as suggested by the works of Devatine [9] and Gerbaud [8]. The vinic acid was considered as a weak acid as suggested by former authors. We assumed that the sensitivity of the dissociation constants of this compound to ethanol was the same as for succinic acid [9]:

$$pK_{1AV} = 3.50 + 1.24 \times 10^{-2}(^\circ\text{alc}) + 1.76 \times 10^{-4}(^\circ\text{alc})^2 \quad (31)$$

$$pK_{2AV} = 5.00 + 1.35 \times 10^{-2}(^\circ\text{alc}) + 2.84 \times 10^{-4}(^\circ\text{alc})^2 \quad (32)$$

A preliminary calculation based on an acid–base titration enables to determine the total molality of this fictitious diacid and its dissociation equilibrium constants. Its total molality was assumed to be constant during the fermentation (i.e. the unmeasured compounds are inert regarding fermentation reactions).

4. Physical properties

4.1. Dissociation constants

The equilibrium dissociation constants are calculated as a polynomial function of the alcoholic degree of the solution [14]:

$$pK = p_0 + p_1(^\circ\text{alc}) + p_2(^\circ\text{alc})^2 \quad (33)$$

The values of p_0, p_1 and p_2 are given for each acid in Table 2.

The pK_a of NH_4^+/NH_3 was taken to 9.2. At the considered pH values, the ammoniac is under the NH_4^+ form.

Table 2
Parameters for dissociation constant calculation of organic acids involved in the study

Acids	K_1			K_2			K_3		
	p_0	p_1	p_2	p_0	p_1	p_2	p_0	p_1	p_2
Lactic	3.89	1.21×10^{-2}	1.5×10^{-4}						
Acetic	4.76	7.96×10^{-3}	2.88×10^{-4}						
Pyruvic	2.72	7.0×10^{-3}	1.61×10^{-4}						
Malic	3.47	1.19×10^{-2}	1.53×10^{-4}	5.10	1.70×10^{-2}	1.09×10^{-4}			
Succinic	4.21	1.24×10^{-2}	1.76×10^{-4}	5.63	1.35×10^{-2}	2.83×10^{-4}			
Citric	3.15	1.17×10^{-2}	1.76×10^{-4}	4.72	1.68×10^{-2}	4.0×10^{-6}	6.41[a]	0	0

$T = 20\,^\circ$C in water–ethanol solution.
[a] $T = 25\,^\circ$C in water [14].

4.2. Density (ρ) and dielectric constant (ε)

Correlations for the density and dielectric constant were determined by Gerbaud [8]. In these correlations, sugar was not taking into account. [23] and [22] proposed a method to calculate the dielectric constant of mixed solvents. New correlations for the density and the dielectric constant were then established taking into account the influence of the sugar (0–200 g L^{-1}) and the ethanol (0–12 °alc) at 20 °C from experimental data of Malmberg and Maryott [15] and from the Handbook of Chemistry and Physics (1999–2000).

$$\rho = 0.998 + 2.799 \times 10^{-4} C_{\text{sugar}} + 1.405 \times 10^{-3} \ (^{\circ}\text{alc}) \quad (34)$$

$$\varepsilon = \varepsilon_0 [71.48 + 0.032 C_{\text{sugar}} + 0.0127 \ (^{\circ}\text{alc})] \quad (35)$$

5. Results and discussion

The model was first validated by comparing the measured pH and the calculated pH of synthetic media at different ethanol contents. Its sensitivity was evaluated on media based on the grape must at different concentrations of sugar, ethanol and acids. Finally, it was compared to pH data obtained during the fermentation of the grape must.

5.1. Model validation on synthetic media

The model was first validated on synthetic media whose composition is perfectly known and close to grape must (Table 3). For each concentration, composition of the must in sugars, alcohol, each organic acids and cations constituted the input of model.

The calculated pH match well the measured pH for three synthetic media at different ethanol content. The relative error on pH is below 1% and the relative error on hydrogen proton below 5%.

5.2. pH simulations for added compounds in the grape must

5.2.1. Validation of pH model including vinic acid calculation

As the overall composition of the grape must is not known, a preliminary calculation determined the fictitious vinic acid molality. It was evaluated to 4.75×10^{-2} mol kg^{-1}. The protonated form is then about 1.5×10^{-2} mol kg^{-1}.

Table 3
Comparison of measured and calculated pH of synthetic media

Components	MS1	MS2	MS3
Glucose (g L^{-1})	200.0	200.0	200.0
Citric acid, H_2O (g L^{-1})	1.518	1.553	1.540
Malic acid (g L^{-1})	6.067	6.085	6.024
$MgSO_4 \cdot 7H_2O$ (g L^{-1})	0.269	0.26	0.268
$CaCl_2$ (g L^{-1})	0.252	0.235	0.233
NaCl (g L^{-1})	0.214	0.228	0.212
Ethanol (°alc)	0	6.0	12.0
pH experimental value	2.35 ± 0.05	2.40 ± 0.05	2.43 ± 0.05
Calculated pH	2.371	2.405	2.447
Relative error on pH (%)	0.89	0.2	0.7
Relative error on m_{H^+} (%)	4.72	1.14	3.84

Table 4
Comparison of the calculated pH and the measured pH according to ethanol content assuming the same influence of ethanol on the vinic acid dissociation constant as for succinic acid

°Alc	pH experimental value	pH calculated value	Relative error on pH (%)	Relative error on m_{H^+} (%)
0	3.290	3.290	0.0	0.0
2	3.315	3.316	0.03	0.23
6	3.375	3.373	0.06	0.46
12	3.470	3.461	0.26	2.09

Experiments with addition of compounds in the grape must solution were carried out (in vitro tests). Simulated values were confronted to experimental values for each added compound.

For sugar concentrations ranging from 0 to 200 g L^{-1}, pH values kept nearly constant (3.23–3.22). Sugars addition modifies the density and the permittivity of the solution without any significant effect on the pH.

For ammonia concentrations ranging from 0 to 112.5 mg L^{-1}, no significant pH deviation was observed.

Organic acids took part in the decrease of the pH since they released H^+ ions by dissociation reaction. Among the organic acids of the grape must, only succinic acid showed a slight influence on the pH with a decrease of 0.01 point for an added concentration of 1 g L^{-1}. The concentration variation of other acids during fermentation is too weak to influence the pH.

Cation concentrations brought positive charges in the solution but do not influence pH value.

Alcohol contributed to a significant rise of the pH. In the must, pH increased of 0.18 point for an ethanol concentration from 0 to 12 °alc (Table 4).

Alcohol influences the density and the permittivity of the medium but its main impact is on the dissociation of the acids. Its influence has been well represented by the model. At high values of ethanol a slight discrepancy between both values can be observed. This is probably caused by the assumption made on the influence of ethanol on the vinic acid dissociation constants.

For succinic acid, results are presented in Table 5 for solutions at 0 and 12 °alc. They show a good agreement between measured and calculated values.

The experimental values are well represented by the model. The organic acids (succinic acid, acetic acid, lactic acid, etc.) are produced in small quantity during alcoholic fermentation. Their influence on the pH is weak at this concentration range but the variation of their dissociation with the ethanol concentration makes it necessary to take them into account in the model.

5.3. pH simulation during a fermentation

5.3.1. Composition of the white grape must and evolution during alcoholic fermentation

The concentration of the main components of the grape must before and after fermentation are given in Table 6. Glucose and fructose, the two sugars in the must, are converted by yeasts into ethanol, CO_2, glycerol and biomass. The nitrogen sources neces-

Table 5
Comparison of the calculated pH and the measured pH in grape must with additional succinic acid concentration and ethanol (0 and 12 °alc)

$C_{succinic\ acid}$ (g L^{-1})	°Alc	pH experimental value	pH calculated value	Relative error on pH (%)	Relative error on m_{H^+} (%)
0	0	3.29	3.291	0	0
0.233		3.29	3.288	0	0
0.411		3.29	3.286	0	0
0.833		3.28	3.28	0	0
0	12	3.47	3.462	0.29	2.33
0.232		3.47	3.459	0.29	2.33
0.411		3.47	3.457	0.29	2.33
0.83		3.46	3.452	0.29	2.33

Table 6
Fermentation medium composition at the beginning and after 173 h of fermentation

Compound	$t = 0$ h	$t = 173$ h
Sugar (g L^{-1})	181.7 ± 4.7	3.8 ± 1.4
Ethanol (g L^{-1})	0.000	98.5 ± 0.65
Glycerol (g L^{-1})	1.61 ± 0.14	7.61 ± 0.64
Dry biomass (g L^{-1})	0.472 ± 0.02	3.939
CO_2 (g L^{-1})	0.000	12.120
Malic acid (g L^{-1})	6.37 ± 0.09	6.37 ± 0.08
Citric acid (g L^{-1})	1.42 ± 0.53	1.42 ± 0.38
Succinic acid (g L^{-1})	0.000	0.71 ± 0.13
Acetic acid (g L^{-1})	0.000	0.55 ± 0.19
Assimilable nitrogen (mg L^{-1})	210.9 ± 0.85	35.9 ± 0.45
Sodium (g L^{-1})	0.0280 ± 0.001	0.0280 ± 0.001
Calcium (g L^{-1})	0.200 ± 0.01	0.200 ± 0.01
Potassium (g L^{-1})	0.768 ± 0.04	0.768 ± 0.04
Magnesium (g L^{-1})	0.090 ± 0.004	0.090 ± 0.004

sary for the yeast growth consist in ammonia and amino acids. The must contains about 210 mg L^{-1} of assimilable nitrogen (74 mg L^{-1} coming from the ammonium ions and 136 mg L^{-1} coming from the alpha amino acids). At the end of the fermentation, 88% of this nitrogen was consumed (i.e. 175 mg L^{-1}).

The grape must contains organic acids. Initially, the most significant are malic and citric acids but they are slightly consumed by yeasts. Organic acids are produced during fermentation. Succinic acid, acetic acid and lactic acid are the most important (Table 6).

Table 7
On line and calculated pH values during fermentation of grape must by *Saccharomyces cerevisiae*

Time (h)	On-line measured pH	Calculated pH	Relative error on pH (%)	Relative error on m_{H^+} (%)
0	3.32	3.324	0.12	0.92
5	3.31	3.314	0.12	0.92
9	3.3	3.307	0.21	1.60
19	3.25	3.251	0.03	0.23
22	3.22	3.247	0.84	6.03
52	3.14	3.178	1.21	8.38
69	3.18	3.208	0.88	6.24
121	3.24	3.274	1.05	7.53
149	3.26	3.295	1.07	7.74
173	3.29	3.31	0.61	4.50

The mineral elements of must (potassium, calcium, sodium and magnesium) are not consumed nor produced during fermentation.

The initial and the fermented grape must contain other compounds which were not taken into account in this study because of their very low concentration. Their contribution to the pH is taken into account through the vinic acid and it is assumed that they were not consumed nor produced during fermentation. Experimental data were validated by checking the carbon element balance. Error did not exceed 10%.

5.3.2. *pH simulation*

The medium composition was determined at different fermentation times. Biomass, Ethanol, assimilable nitrogen and pH evolution was represented in Fig. 1.

Regarding pH evolution, fermentation can be divided into two phases: during the first 50 h, the pH decreases from 3.32 to 3.14, then it increases up to 3.29 during the 100 following hours. The pH decrease is assumed to be correlated to the nitrogen consumption. The nitrogen concentration did not influence the pH itself when it was added in the must. However, it is known that during the fermentation, the consumption of nitrogen by yeasts produces H^+ ions. Indeed, Castrillo et al. [16] showed that the assimilation of one ammonium mole by yeasts leads to the release of one H^+ mole in solution. Won et al. [17], Kotyk [18] and Sigler et al. [19] also mentioned the same phenomenon. Studies of Hernandez-Orte et al. [20] showed that the main part of the nitrogen source was consumed between 0 and 50 h of alcoholic fermentation. In their work, assimilable nitrogen decreases from 231 to 25.6 mg L^{-1} during this period. We

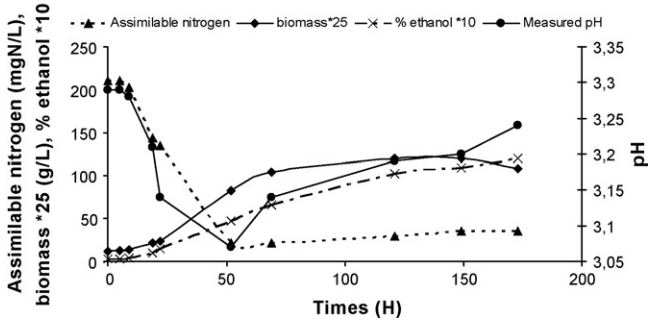

Fig. 1. Assimilable nitrogen, biomass, % ethanol and pH evolution during fermentation.

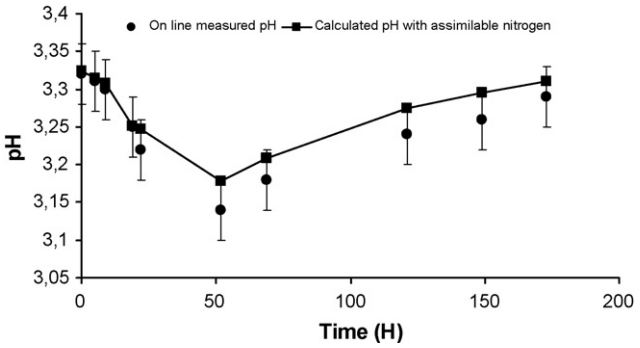

Fig. 2. Comparison of the evolution of the pH calculated and measured during the alcoholic fermentation of the white grape by *Saccharomyces cerevisiae* QA-23.

find similar results with an assimilable nitrogen content decrease from 210 to 35.6 mg L^{-1}. Between 40 and 160 h of fermentation, the ethanol concentration increases in the medium which can explain the increase of pH during this period.

Experimental and calculated pH values are plotted in Fig. 2. During the first 50 h, it was assumed that, for each mole of assimilable nitrogen consumed (ammonium and amino acids), one mole of H^+ was released in solution. Under this assumption, the model is in good agreement with the experimental values of the pH. The highest discrepancy between experimental and calculated values, observed after 52 h, represents only 0.038 pH unit i.e., a relative error of 1.2% This error could be considered as non-significant, but it is about 10 times higher than the values previously found (Table 7). The assumption made on the H^+ release associated to nitrogen consumption should be studied in more detail to improve the representation of pH during this phase. If the literature is clear for ammonium consumption, a few number of studies were realised for the effect on pH evolution of amino acids consumption. The assumptions made on the vinic acid may also lead to underestimate the dissociation of the medium components (buffer effect) and so the H^+ release.

6. Conclusion

A model to predict pH from the measurement of the main compounds of the fermentation medium has been developed. An original formulation of the model by writing mass balance on invariant elements enables a simplified resolution procedure. No dissociation reaction extend has to be initialized. The model was validated on synthetic media and grape must without tartaric acid. Its reliability regarding the influence of different compounds (sugar, alcohol) and phenomena (assimilation of ammonium ions) was proved through a fermentation carried out on a grape must. The model must be now extended to take into account the complexation phenomena. To study the opportunity of controlling fermentation with pH further, this model will be coupled to a stoichio-kinetic model representing the reaction occurring during the fermentation.

Appendix A. Nomenclature

$^\circ$alc	alcoholic degree
a_{H+}	activity of ion H^+ (mol kg^{-1} of solution)
A_IH	monoacid
$A_{II}H_2$	diacid
$A_{III}H_3$	triacid
C_i	mass concentration of compound i (g L^{-1})
e	electron charge (1.6021177E − 19 C)
I	ionic force of the solution (mol kg^{-1} of solution)
K_j	constant of the jth acid dissociation
K_w	water dissociation constant
K_β	Boltzmann constant (1.380658E − 23 J K^{-1})
m_i	molality of compound i (mol kg^{-1} of solution)
$m^T{}_i$	total molality of the specie i
N_A	Avogadro number (6.022136×10^{23} mol^{-1})
T	temperature (K)
Z_i	charge of the electrolyte

Greek symbols

$\gamma_\pm$	average activity coefficient
γ_{H^+}	H^+ ion activity coefficient
ε	Dielectric constant of the solution
ε_0	permittivity of vacuum (8.854187E-12 F m^{-1})
ρ	density of solution (kg L^{-1})

References

[1] V. Renouf, A. Lonvaud-Funel. Le suivi microbiologique du vin. Partie 1: De la parcelle au conditionnement: un outil pour une œnologie raisonnée. Revue des œnologues No. 118, 2006a.

[2] V. Renouf, A. Lonvaud-Funel. Le suivi microbiologique du vin. Partie 2: Conseils pratiques pour la mise en place d'un suivi microbiologique. Revue des œnologues No. 119, 2006b.

[3] J.M. Barandica, A. Santos, D. Marquina, F. López, F.J. Acosta, J.M. Peinado, A mathematical model for toxin accumulation by killer yeasts based on the yeast population growth, J. Appl. Microbiol. 86 (5) (1999) 805.

[4] S.A. Lemmel, R.C. Heimsch, R.A. Korus, Kinetics of growth and amylase production of *Saccharomycopsis fibuligera* on potato processing wastewater, Appl. Environ. Microbiol. 39 (2) (1980) 387–393.

[5] C.R. Righelato, D. Rose, A.W. Westwood, Kinetics of ethanol production by yeast in continuous culture, Biotechnol. Lett. 3 (1) (1981) 3–8.

[6] D. Vasic-Racki, U. Kragl, D. Conrad, C. Wandrey, Modelling of yeast alcohol dehydrogenase catalysed production of chiral alcohols, Chem. Biochem. Eng. Quart. 12 (2) (1998) 87–95.

[7] B. Ratsimba. Cristallisation du bitartrate de potassium à partir de solutions hydroalcooliques—Extensions des résultats à l'œnologie. Thèse de doctorat de l'INP, 1990.

[8] V. Gerbaud. Effet des polysaccharides sur la cristallisation du bitartrate de potassium en solutions hydroalcooliques et dans les vins. Thèse de doctorat à l'INP (1996).

[9] A. Devatine. Maîtrise de l'acidité des vins: Désacidification par précipitation de malates de calcium et simulation des équilibres physico-chimiques a l'aide du logiciel MEXTAR, thèse de doctorat de l'INP, 2002.

[10] G.L. Miller, Use of dinitrosalysilic reagent for determination of residual sugar, Anal. Chem. 31 (1959) 426–428.

[11] C.E. Harvie, N. Moller, J.H. Weare, The prediction of mineral solubility in natural waters: the Na-K-Mg-Ca-H-Cl-SO_4-HCO_3-CO_3-CO_2-H_2O system to high ionic strengh at 25 °C, Geochemica et Cosmochemica Acta 48 (1984) 723–751.

[12] R. Aris. Elementary chemical reactor analysis. Ed. Butterworths reprint Series in chemical engineering, 1989.
[13] B. Carnahan, H.A. Luther, J.O. Wilkes, Applied Numerical Methods, Krieger Publishing Company, 1969.
[14] L. Usseglio-Tomasset, P.D. Bosia. Determinazione delle costanti di dissociatione dei principali acidi del vino in soluzioni idroalcoliche di interesse enologico, Rivisto di viticoltura e di enologia di conegliano, 31 (1978) 380–403.
[15] M.S. Malmberg, A.A. Maryott, Dielectric constant of water from 0° to 100 °C, J. Res. Natl. Bur. Stand. 56 (1956) 1–8.
[16] J.I. Castrillo, I. DE Miguel, U.O. Ugalde, Proton production and consumption pathways in yeast metabolism. A chemostat culture analysis, Yeast 11 (1995) 1353–1365.
[17] J.I. Won, Y.L. Yang, B.G. Kim, C.Y. Choi, Adaptative control of specific growth rate based on proton production in anaerobic fed-batch culture, Biotechnol. Lett. 15 (5) (1993) 511–516.
[18] A. Kotyk, Proton extrusion in yeast, in: S. Fleischer, B. Fleischer (Eds.), Method in Enzymology, vol. 174, Academic Press, New York, 1989, pp. 592–603 (Biomembranes, Part U).
[19] K. Sigler, A. Knotkova, A. Kotyk, Factor governing substrate induced generation and extrusion of proton in yeast, Biochim. Biophys. Acta 643 (1981) 572–582.
[20] P. Hernandez-Orte, M.J. Ibarz, J. Cacho, V. Ferreira, Addition of acids to grape juice of merlot variety: effect on amino acid uptake and aroma generation during alcoholic fermentation, Food Chem. 98 (2006) 300–310.
[21] K. Denbigh, The Principles of Chemical Equilibrium, With Applications in Chemistry and Chemical Engineering, 4th ed., Cambridge University Press, Cambridge, 1981, p. 494.
[22] D. Dumanovic, D.J. Kosanovic, D. Arkakovic, J. Jovanovic, Solubilization of ipronidazole by co-solvents, Pharmazie 47 (1992) 603–607.
[23] S. Prakongpan, T. Nagai, Solubility of acetaminophen in cosolvents, Chem. Pharm. Bull. 32 (1984) 340–343.