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Improved Performances and Control of Beer Fermentation Using Encapsulated α-Acetolactate Decarboxylase and Modeling

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The use of the enzyme α-acetolactate decarboxylase allows the acceleration of beer fermentation/maturation because it shunts diacetyl formation, whose elimination is the rate-limiting step of the process. To obtain a cost reduction by using this exogenous enzyme, we propose a new process involving recoverable encapsulated α-acetolactate decarboxylase. The performance of traditional and new processes was investigated by a modeling approach. A simple model, focused on α-acetolactate and diacetyl profiles during beer fermentation, was set up. The simulated profiles are consistent with literature data. This study shows also that encapsulated α-acetolactate decarboxylase allows the acceleration of beer fermentation as efficiently as free α-acetolactate decarboxylase. The advantage of immobilized α-acetolactate decarboxylase versus free enzyme is that it is recoverable and reusable, which means a process cost reduction.

Introduction

The presence of diacetyl is highly undesirable for most beers as it gives to the beer a very unpleasant flavor (odor and taste of butterscotch, buttermilk), even in concentrations as low as 0.15 ppm (*1*).

During beer fermentation, the yeast excretes α-acetolactate as an intermediate of amino acids leucine and valine synthesis, which is essential for yeast growth. The α-acetolactate is then converted into diacetyl by a chemical reaction that is *slow* (*2*). Diacetyl is then transformed into acetoin by an enzymatic reaction. Acetoin has a much higher flavor threshold (50 ppm, giving a fruity, moldy, and woody flavor (*1*)) and does not cause any off-flavor to the beer. The rate-limiting step of fermentation is the conversion of α-acetolactate into diacetyl. Consequently, beer maturation is long (2−12 weeks, depending on the type and temperature (*3*)). Today, in process control, the level of diacetyl in beer is used as a tool for supervision, particularly as a signal of the end of warm maturation (*3*).

Figure 1 gives an overview of the brewing fermentation process. In a traditional process, primary fermentation and maturation are conducted in batch (Figure 1a). The majority of yeast is removed by sedimentation or centrifugation after primary fermentation. Primary fermentation can also be made in an immobilized yeast reactor, allowing an accelerated fermentation. In some of immobilized yeast system processes, primary accelerated fermentation is stopped after 1 or 2 days and is followed by a traditional maturation step (*4*). In such cases, the time to eliminate diacetyl remains long, reducing strongly the advantage of accelerated primary fermentation. Another process of accelerated fermentation consists of heating the green beer to 60−90 °C for 5−60 min (*5*−*7*) before the maturation step (Figure 1b). The aim of heating is to quickly convert α-acetolactate into diacetyl/acetoin. However, this heating is detrimental to the beer flavor and has to be performed in the absence of oxygen and yeast.

The use of the enzyme α-acetolactate decarboxylase has been proposed to overcome the diacetyl formation (*8*). This enzyme converts α-acetolactate directly into acetoin simultaneously to the chemical transformation of α-acetolactate into diacetyl (Figure 2). The use of α-acetolactate decarboxylase makes it possible to shorten beer primary fermentation (*9*−*11*) until no more maturation is needed in regard to diacetyl (*4*).

The present study is related to an evaluation of α-acetolactate decarboxylase encapsulation in small spheres of polyelectrolyte complexes (*12*). The use of encapsulated α-acetolactate decarboxylase during primary batch fermentation makes it possible to accelerate the total time of beer production. The advantage of immobilized versus soluble α-acetolactate decarboxylase is the recoverery of the enzyme after fermentation; its reuse reduces the cost of exogenous enzyme (Figure 1c). Immobilized yeast produce more α-acetolactate than what is observed during classical fermentation (Figure 1a), and the green beer needs to be heated before a maturation step (*5*, *6*). Since α-acetolactate decarboxylase transforms α-acetolactate as soon as it appears, we propose a combined process using both immobilized yeast and encapsulated α-acetolactate decarboxylase (Figure 1d and Figure 3). Using encapsulated α-acetolactate decarboxylase should be a substitute for the heating step of the accelerated process.

The aim of this study was to investigate the application of encapsulated α-acetolactate decarboxylase by a modeling approach. The first studies concerning process engi-

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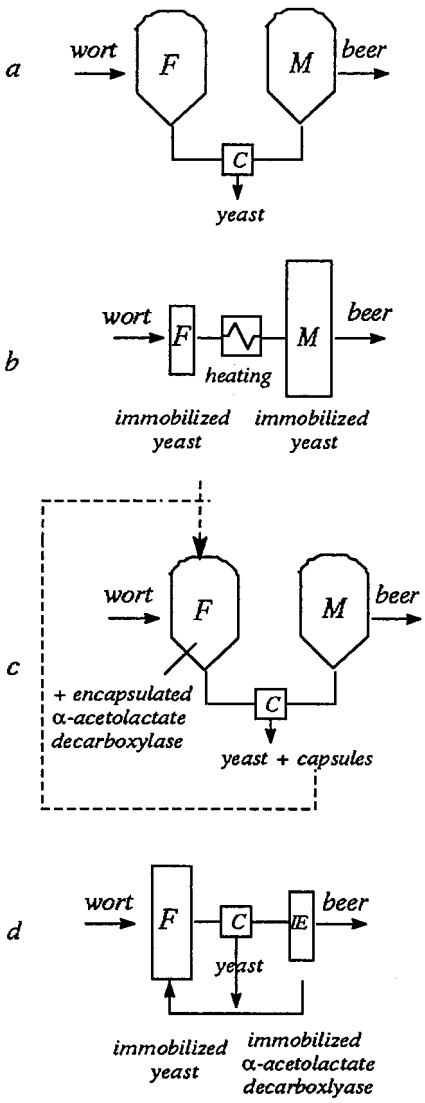

Figure 1. Beer fermentation processes. (a) Classical fermentation process (batch): F, primary fermentation; M, maturation; C, centrifuge. (b) Batch fermentation process using yeast *and* encapsulated α-acetolactate decarboxylase. (c) Immobilized yeast system for primary fermentation (pilot scale). Industrially, immobilized yeast is only applied for beer maturation (*6*). (d) New process using immobilized yeast *and* immobilized α-acetolactate decarboxylase system (IE).

neering of beer fermentation were conducted by some American authors in the 1960s (*13*−*16*). Many authors have brought their contribution to the modeling of beer fermentation since the 1980s (*17*−*20*). As usual in food technology, the final product is of complex composition. Therefore, the proposed models imply numerous variables and intricate equations. Consideration of the biochemical pathways separately seems more appropriate for accuracy of simulation: diacetyl (*21*), esters (*22*), fusel alcohols (*23*), or flavors (*24*).

The present paper deals with simulation and study of biochemical changes leading to α-acetolactate and diacetyl formation with a simple system of equations, that differs from usual modeling studies (*25*). Performances of encapsulated α-acetolactate decarboxylase were also investigated using the established model.

Aim of Modeling

Modeling of α-acetolactate and diacetyl profiles during beer fermentation is mainly directed to predict the time needed to remove diacetyl. The presence of α-acetolactate decarboxylase, as free enzyme or encapsulated in small spheres, is taken into account in the model in order to show its impact on the fermentation profile.

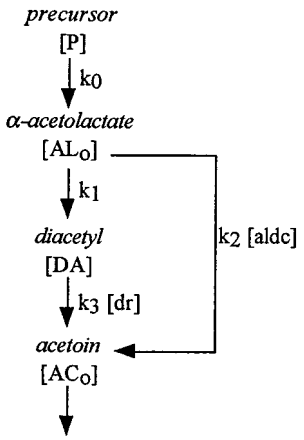

Figure 2. Formation of diacetyl in beer and action of α-acetolactate decarboxylase: aldc, α-acetolactate decarboxylase concentration (mg/L); dr, diacetyl reductase concentration (mg/L).

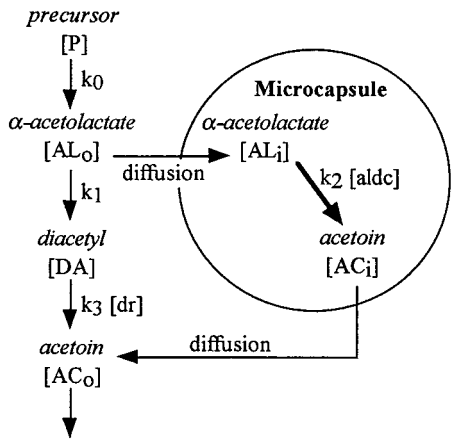

Figure 3. Conversion of α-acetolactate by encapsulated α-acetolactate decarboxylase: aldc, α-acetolactate decarboxylase concentration (mg/L); dr, diacetyl reductase concentration (mg/L); P, precursor of α-acetolactate (μM); AL_o, α-acetolactate outside the capsules (μM); AL_i, α-acetolactate inside the capsules (μM); DA, diacetyl (μM); AC_o, acetoin outside the capsules (μM); ACi, acetoin inside the capsules (μM).

One major concern of this study is to establish whether the structure of the spheres (diameter, thickness of the membrane) allows sufficient mass transfer for conversion of α-acetolactate into acetoin. Modeling has been built up with regard to polyelectrolyte complex capsules (1 mm diameter, 10 μm membrane thichness). However, more efficiently performing 600−800 μm polyelectrolyte complex capsules have been obtained with α-acetolactate decarboxylase (*25*).

Accelerated fermentations obtained with immobilized yeast systems (*4*, *6*) have been simulated. The impact of the presence of encapsulated α-acetolactate decarboxylase on "accelerated fermentation" (i.e., immobilized yeast systems) has also been tested.

Structure of the Model

Model with Free α-Acetolactate Decarboxylase. Figure 2 gives the structure of the reactions involved in the model. This model focuses on three main compounds: α-acetolactate precursor (P), α-acetolactate (AL), and diacetyl (DA). Previous works (*17*, *26*) have shown that α-acetolactate is formed from glucose (i.e., total

fermentable sugar). α-Acetolactate is converted into diacetyl by spontaneous oxidative decarboxylation or, in the presence of α-acetolactate decarboxylase, directly into acetoin. Diacetyl is transformed into acetoin via diacetyl reductase enzyme present in the yeast.

Conversion of α-acetolactate precursor into α-acetolactate may be described by a first-order reaction (with respect to a constant in inverse of time, d^{-1}):

$$\frac{d[P]}{dt} = -k_0[P] \quad (1)$$

α-Acetolactate concentration results from mass balance between precursor transformation (described above) and its decarboxylation into diacetyl (first-order kinetic). In the presence of α-acetolactate decarboxylase (aldc), α-acetolactate is also enzymatically converted into acetoin (Michaelis kinetics):

$$\frac{d[AL]}{dt} = k_0[P] - k_1[AL] - \frac{V_{m1}}{K_{m1} + [AL]}[aldc][AL] \quad (2)$$

Diacetyl, which is formed from oxidative decarboxylation of α-acetolactate, is transformed into acetoin via diacetyl reductase enzyme (Michaelis term proportional to diacetyl reductase concentration [dr]):

$$\frac{d[DA]}{dt} = k_1[AL] - \frac{V_{m2}}{K_{m2} + [DA]}[dr][DA] \quad (3)$$

During fermentation of beer, α-acetolactate concentration ([AL]) reaches an order of magnitude of 10 μM (*17*). The Michaelis constant of α-acetolactate decarboxylase experimental determination shows that $K_{m1} \gg [AL]$ (see Determination of Rate Constants), so

$$\frac{V_{m1}}{K_{m1} + [AL]} \approx \frac{V_{m1}}{K_{m1}}$$

Galzy and co-workers in 1983 (*27*) estimated the Michaelis constant of diacetyl reductase (from *Saccharomyces uvarum*) equal to $K_{m2} = 30$ mmol/L. The order of magnitude of diacetyl during beer fermentation is 10−20 μmol/L (*28*), so here too $K_{m2} \gg [DA]$ and

$$\frac{V_{m2}}{K_{m2} + [DA]} \approx \frac{V_{m2}}{K_{m2}}$$

From these observations the system becomes:

$$\frac{d[P]}{dt} = -k_0[P] \quad (4)$$

$$\frac{d[AL]}{dt} = k_0[P] - k_1[AL] - k_2[aldc][AL] \quad (5)$$

$$\frac{d[DA]}{dt} = k_1[AL] - k_3[dr][DA] \quad (6)$$

where $k_2 = V_{m1}/K_{m1}$ and $k_3 = V_{m2}/K_{m2}$.

Model with Encapsulated α-Acetolactate Decarboxylase. Preliminary studies have shown that encapsulation does not affect the kinetic behavior of α-acetolactate decarboxylase upon time (activity measurements) (*25*, *35*). However in the presence of encapsulated α-acetolactate decarboxylase, mass transfer has to be taken into account (Figure 3).

Transfers inside and outside of the microcapsule are assumed to be described by Fick's Law. α-Acetolactate transfer is governed by the acetolactate concentration gradient through the microcapsule membrane and is proportional to the permeability of the capsule membrane. Permeability is equal to the diffusion coefficient of the membrane (D_e) multiplicated by interfacial area (A) and divided by thickness (δ) of the membrane. No concentration profile is expected inside the capsules. As the capsules are quite flexible, external turbulence must create a mixing inside the microcapsules. In the case of encapsulated α-acetolactate decarboxylase, the mass balance of α-acetolactate must consider both external and internal α-acetolactate concentrations ($[AL_o]$ and $[AL_i]$, respectively) leading to eqs 8 and 9. Equation 8 results from the precursor transformation, conversion of α-acetolactate into diacetyl, and diffusion of α-acetolactate in microcapsules. Equation 9 describes the α-acetolactate diffused in the microcapsules and the enzymatic conversion of α-acetolactate into acetoin.

Thus the set of eqs 4−6 becomes:

$$\frac{d[P]}{dt} = -k_0[P] \quad (7)$$

$$\frac{d[AL_o]}{dt} = k_0[P] - k_1[AL_o] - \frac{D_e \cdot A}{\delta}([AL_o] - [AL_i]) \quad (8)$$

$$\frac{d[AL_i]}{dt} = \frac{D_e \cdot A}{\delta \cdot \alpha}([AL_o] - [AL_i]) - k_2[aldc][AL_i] \quad (9)$$

$$\frac{d[DA]}{dt} = k_1[AL_o] - k_3[dr][DA] \quad (10)$$

where α is the volume of microcapsules per liter of bioreactor, and A is the interfacial microcapsule area. Microcapsules are considered as spheres, so that interfacial area is given by

$$A = \frac{\alpha \cdot \pi \cdot d^2}{\frac{\pi}{6} d^3} \quad (11)$$

so

$$A = \frac{6\alpha}{d} \quad (12)$$

Model with Immobilized Yeast System. Beer fermentation involving immobilized yeast systems is hastened by the high cell density and also by changes in yeast metabolism due to immobilization (*29*−*33*). To simulate this acceleration, the same equation system can be used, but the two yeast-dependant reaction constants, formation of α-acetolactate (k_0) and consumption of diacetyl (k_3), are increased. Other constants are kept at previously mentioned values for the first 7 days of primary fermentation.

Determination of Rate Constants and Initial Value of Variables

Values of rate constants and initial values of variables were either determined experimentally or taken from literature (Table 1). As constants depend on temperature and as primary fermentation and maturation are conducted at two different temperatures, constants take two values, one for each temperature.

Rate of Sugars Consumption. The synthesis rate constant of 4-acetolactate (k_0) is given by linear regres-

Table 1. Model Constants and Values

parameter	units	value: first 7 days of fermentation 12−15 °C	value: after 7th day of fermentation 0 °C	reference
k_0	d^{-1}	0.34	0.11	
k_1	d^{-1}	0.54	0.12	
k_2	L/(d mg)	0.34	0.19	
k_3·[dr]	d^{-1}	0.4	0.28	*28*, *36*
A	m^{-1}	60	60	
d	m	10^{-3}	10^{-3}	*25*
D_e	m^2/s	10^{-10}	10^{-10}	*37*
α	% (v/v)	1	1	
δ	m	10^{-5}	10^{-5}	*25*

sion of the first-order representation of total fermentable sugars consumption during the primary fermentation of beer (Figure 4). This gives $k_0 = 0.34$ d^{-1} for a fermentation at 14 °C (primary fermentation). The value of k_0 at 0 °C, the normal temperature of beer at the end of maturation ($k_0 = 0.11$ d^{-1}) was calculated under the assumption that all of the enzymatic reactions involved during primary fermentation were slowed in the same range as diacetyl reductase during maturation (see Kinetic Parameters of Diacetyl Reductase).

Decarboxylation of α-Acetolactate. The rate of oxidative decarboxylation of α-acetolactate was measured as follows. Freshly synthesized α-acetolactate in degassed beer at pH 4.2 was analyzed for diacetyl and acetoin concentrations by spectrophotometric assay (*34*) as a function of time, during at least 3 h at different temperatures. Slopes of the obtained straight lines give the rate of decarboxylation. As shown in Figure 5, temperature has a strong effect on decarboxylation rate. The values introduced in the model for decarboxylation rate (k_1) are: $k_1 = 0.54$ d^{-1} during the first 7 days of fermentation (primary fermentation, 12−15 °C) and $k_1 = 0.12$ d^{-1} for secondary fermentation (0 °C).

Kinetic Parameters of α-Acetolactate Decarboxylase. Kinetic parameters of α-acetolactate decarboxylase have been measured at 10 °C using a spectrophotometric assay (*35*). Initial rate constants were plotted on a Lineweaver−Burk graph as a function of inverse of α-acetolactate concentration (Figure 6). From this graph, apparent maximum rate and Michaelis constants (respectively V'_{max} and K'_{max}) were calculated:

$$V'_{max} = 0.65\ \mu\text{mol/min mg}$$

$$K'_{max} = 2750\ \mu\text{mol/L}$$

For modeling purpose, the conversion of α-acetolactate into acetoin by α-acetolactate decarboxylase was described by a specific rate constant, k_2, estimated by

$$k_2 = \frac{V'_{max}}{K'_{max}} \qquad (13)$$

which gives $k_2 = 2.3\ 10^{-4}$ L/min mg or 0.34 L/d mg.

Kinetic Parameters of Diacetyl Reductase. In the absence of sufficient data in the literature, the parameters related to diacetyl reductase were estimated on the basis of two clues: the higher level of diacetyl reached (at peak) and the time of diacetyl peak apparition. Different values were tested in the model while other parameters were kept constant. By comparison with literature data, for the diacetyl peak between 5 and 7 days, at a level of 10−20 μM (*28*), the obtained value is k_3[dr] = 0.4 d^{-1}. For the maturation period, this coefficient was lowered to 30% according to van den Berg's team observations: k_3[dr] = 0.12 d^{-1} (*36*), see eqs 6 and 10.

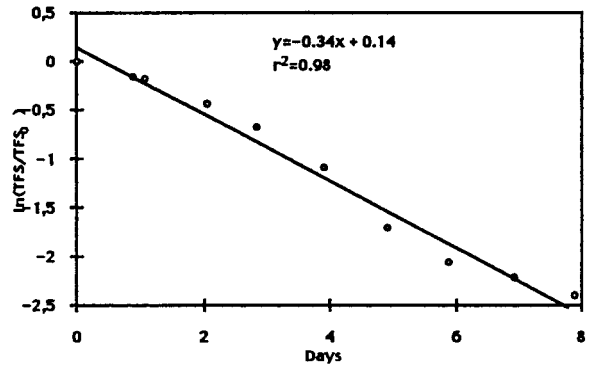

Figure 4. First-order representation of total fermentable sugars (TFS) consumption during primary fermentation (data taken from ref 17).

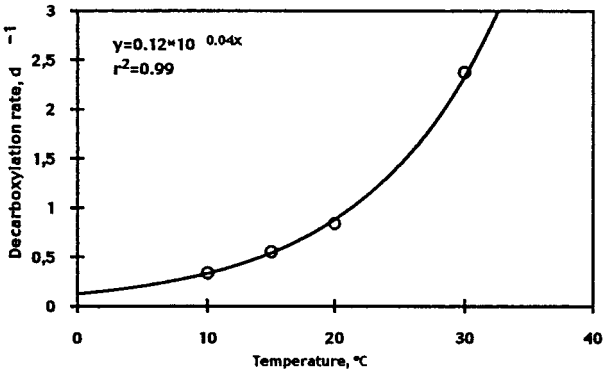

Figure 5. Spontaneous decarboxylation rate of α-acetolactate decarboxylase as a function of temperature: S, α-acetolactate decarboxylase concentration; *v*, initial rate constants, enzymatic decarboxylation of α-acetolactate.

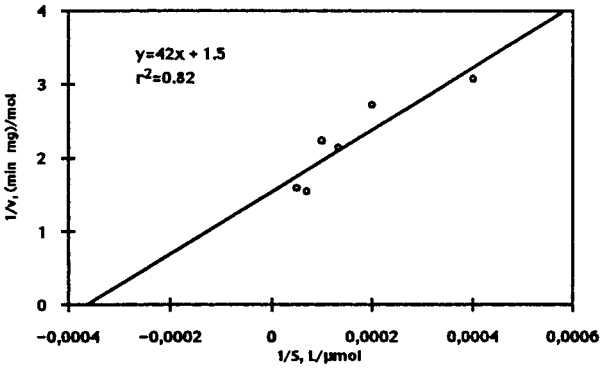

Figure 6. Linewaever−Burk plot for α-acetolactate decarboxylase.

Other Constants. The diffusion coefficient of α-acetolactate, diacetyl, and acetoin through capsule membrane (D_e) had to be estimated. The diffusion coefficient of the closest compound and membrane cited in the literature was chosen as an order of magnitude of D_e: 10^{-10} m^2/s, by analogy with diffusion coefficient of glucose in gels (*37*). Capsules diameter (d) is equal to 1 mm, and membrane thickness (δ) is 10^{-5} m (data from microscopic observations (*25*)). Concentration of capsules in the reactor was set to 1%. The temperature diagram for simulated fermentation was considered to be 14 °C for the first 7 days, then 0 °C for maturation, until the diacetyl concentration becomes lower than its flavor

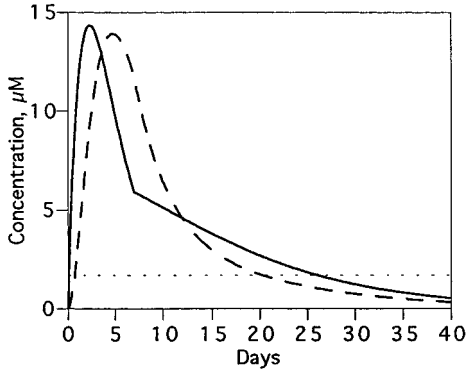

Figure 7. Simulated concentrations of α-acetolactate and diacetyl during a traditional fermentation of beer: (—) α-acetolactate; (– –) diacetyl; (• • •) sensory threshold of diacetyl (1.7 μm).

threshold. The flavor thresholds of added diacetyl into beer are between 0.08 and 0.15 ppm (0.93 and 1.7 μM) (*1*, *38*).

Initial Value of Variables. At the beginning of fermentation, concentrations of α-acetolactate and diacetyl are equal to zero. Initial concentration of α-acetolactate precursor was estimated by iteration test regarding the simulated α-acetolactate peak level and date obtained. With 50 μmol/L of precursor the obtained peak level (10−20 μM) and date (2−3 days) were consistent with literature data (*17*).

Results of the Simulation

The equation systems described previously (eqs 7−10) were introduced in a MathcadPlus software (MathSoft Inc., Cambridge, MA) file, and calculations were conducted with a variable step Runge−Kutta integration (printed version of the files are available upon request to the corresponding author). Simulations were established for different cases:

- batch "traditional" fermentation of beer,
- batch fermentation of beer containing free α-acetolactate decarboxylase,
- batch fermentation of beer containing encapsulated α-acetolactate decarboxylase,
- accelerated fermentation (such as immobilized yeast system), and
- accelerated fermentation using encapsulated α-acetolactate decarboxylase.

Formation of Diacetyl in a Batch Fermentation. Simulation of α-acetolactate and diacetyl during beer fermentation is shown in Figure 7. Both compounds reach a peak and then slowly decrease. The α-acetolactate peak occurs after 3 days of fermentation with a value of 1.8 ppm (14 μmol/L), and the diacetyl peak on the sixth day has a value of 1.2 ppm (14 μmol/L). Those results are consistent with the few published literature data (*17*, *21*, *28*). In fact, limited data are available in the literature because most brewers follow the total diacetyl level, which is the sum of α-acetolactate, acetoin, and diacetyl concentrations. Time needed for diacetyl to become lower than 1.7 μM is approximately 20 days. This duration is very similar to the usual time of beer fermentation. The batch fermentation profile is taken as a reference for comparison for subsequent experiments.

Formation of Diacetyl in a Batch Fermentation, with α-Acetolactate Decarboxylase. The model was applied for a fermentation in the presence of 2 mg/L of free α-acetolactate decarboxylase. The impact of free α-acetolactate decarboxylase on α-acetolactate and diacetyl formation is shown in Figure 8. α-Acetolactate and diacetyl peaks are located at 2 and 4 days. Maximum concentration values are significantly lower than the reference batch: around 1 ppm (8 μM) for α-acetolactate and 0.6 ppm (7 μM) for diacetyl. As a consequence, the time required to reduce the diacetyl level below its threshold is shortened to 12 days. This corresponds to a reduction of 8 days compared with a traditional fermentation.

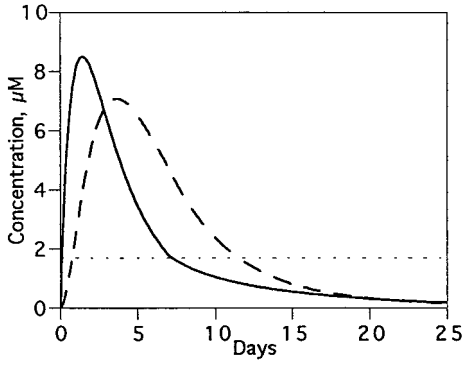

Figure 8. Simulated concentrations of α-acetolactate and diacetyl during fermentation of beer with 2 mg/L of free α-acetolactate decarboxylase: (—) α-acetolactate; (– –) diacetyl; (• • •) sensory threshold of diacetyl (1.7 μm).

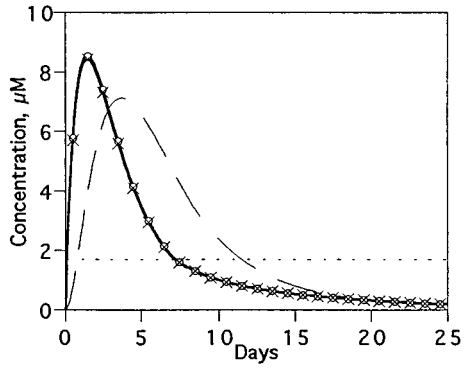

Figure 9. Simulated concentrations of α-acetolactate and diacetyl during fermentation of beer with 2 mg/L of encapsulated α-acetolactate decarboxylase: (○) α-acetolactate inside the capsules; (×) α-acetolactate outside the capsules; (– –) diacetyl; (• • •) sensory threshold of diacetyl (1.7 μm).

As a comparison, Jepsen's Danish team has conducted some trials on beer fermentation in the presence of α-acetolactate decarboxylase in similar conditions (*10*, *39*). On full-scale trials with a fermentation diagram containing a diacetyl rest period (4 days at 14 °C), addition of equivalent of 0.9 mg/L α-acetolactate decarboxylase permitted the gain of 3 or 4 days on beer fermentation depending on the brewery. Our results are consistent with these data.

Formation of Diacetyl in a Batch Fermentation, with Encapsulated α-Acetolactate Decarboxylase. As mentionned before, the aim of this model was to evaluate the potential of encapsulated α-acetolactate decarboxylase during beer fermentaton. This would be demonstrated if *encapsulated* α-acetolactate decarboxylase accelerates beer fermentation as well as free α-acetolactate decarboxylase.

α-Acetolactate and diacetyl profiles obtained with encapsulated α-acetolactate decarboxylase during beer fermentation are shown in Figure 9. As indicated in Figure 3, two locations have to be considered for α-acetolactate (external and internal) because of the partitioning due to microcapsules. Transfer calculations lead

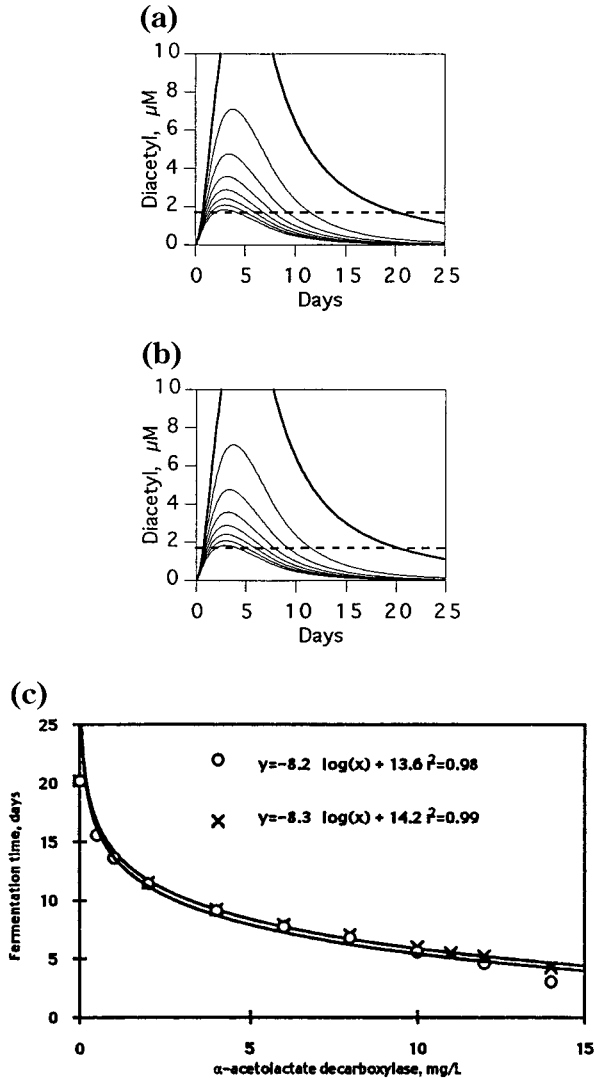

Figure 10. Simulated fermentation time as a function of α-acetolactate decarboxylase added: (a,b) Diacetyl profiles of simulated fermentation involving free (a) or encapsulated (b) α-acetolactate decarboxylase. Curves from top to bottom refer to 0, 2, 4, 6, 8, 10, 12, and 14 mg/L of α-acetolactate decarboxylase. (c) Simulated fermentation time as a function of α-acetolactate decarboxylase added: (○) free α-acetolactate decarboxylase; (×) encapsulated α-acetolactate decarboxylase.

to very slight variations between internal and external levels of α-acetolactate concentrations (see curves with crosses and circles in Figure 9). Differences between diacetyl levels in the presence of free and encapsulated α-acetolactate decarboxylase have been calculated for each point of the graph, and they are always less than 2% (data not shown).

The time needed for diacetyl to be consumed is 12 days as shown in previous results (Figure 8). So the gain in time compared to a traditional batch fermentation of beer is the same with encapsulated as it is with free α-acetolactate decarboxylase, i.e., 8 days. If we consider that a traditional batch fermentation lasts 22−28 days ("theoretical" duration, assuming 7 days for primary fermentation, followed by 15−21 days of maturation), 8 days corresponds to a gain in productivity of 30−35%.

Moreover, encapsulation of α-acetolactate decarboxylase does not limit the performance in regard to fermentation profile. Indeed, transfer is related to concentration gradient, and so to the distance between outside and the core of the capsule and to the thickness of membrane.

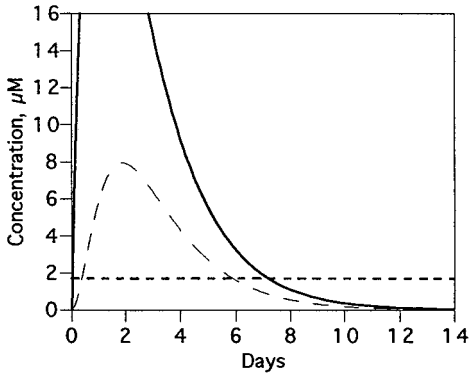

Figure 11. Simulated concentrations of α-acetolactate and diacetyl during accelerated fermentation of beer (immobilized yeast system): (—) α-acetolactate; (− −) diacetyl; (· · · ·) sensory threshold of diacetyl (1.7 μm).

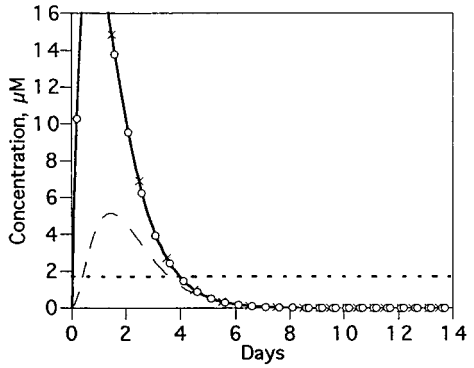

Figure 12. Simulated concentrations of α-acetolactate and diacetyl during accelerated fermentation of beer with 2 mg/L of encapsulated α-acetolactate decarboxylase: (○) α-acetolactate inside the capsules; (×) α-acetolactate outside the capsules; (− −) diacetyl; (· · · ·) sensory threshold of diacetyl (1.7 μm).

The small size of the spheres, as well as the low thickness of the membrane, can explain those very good results.

Fermentation Time versus α-Acetolactate Decarboxylase Concentration. Impact of quantity of α-acetolactate decarboxylase added to the fermenting beer is of interest for cost calculation. The aim of use of this exogenous enzyme is to shorten fermentation time. However, a balance between saved costs and cost due to exogenous enzyme might be considered.

Fermentation kinetics expressed in term of diacetyl concentration profiles obtained at different α-acetolactate decarboxylase concentrations (free or encapsulated) are presented in Figures 10. Graph 10a is for free enzyme, and graph 10b is for immobilized enzyme. Graph 10c was obtained with the results of graphs 10a and 10b. As expected, the more enzyme (is added), the lower (is) the diacetyl concentration profile. Consequently, the time needed to get a diacetyl level lower than 1.7 μM decreases with the enzyme concentration. At enzyme concentration of 14 mg/L or more, diacetyl never gets higher than 1.7 μM. This is due to the strong conversion of α-acetolactate into acetoin catalyzed by the enzyme. However, 8 mg of α-acetolactate decarboxylase per liter is sufficient to avoid a maturation step in regard to diacetyl, because diacetyl concentration is below its threshold at the end of primary fermentation.

Formation of Diacetyl during Accelerated Fermentation with Immobilized Yeast System. Figure 11 shows simulated α-acetolactate and diacetyl profiles during an accelerated fermentation. Trials to determine the value of the multiplication factor were processed (whole numbers between 1 and 5, results not shown).

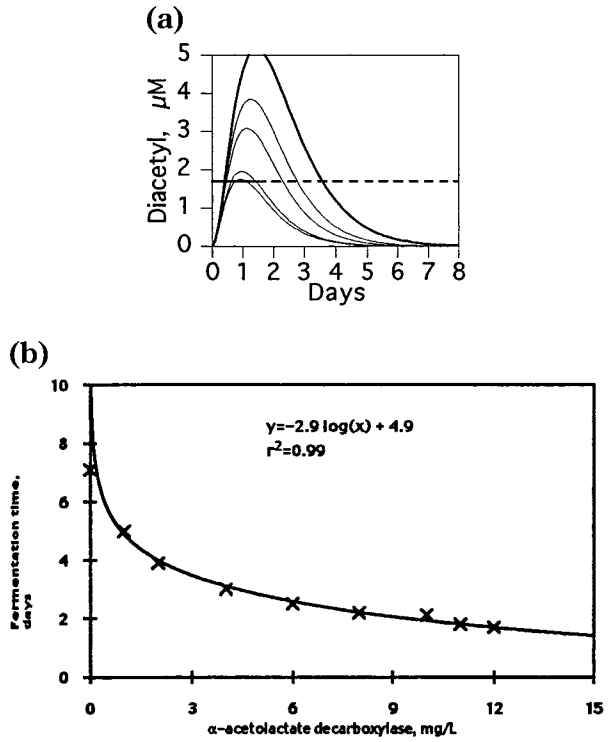

Figure 13. Simulated accelerated fermentation time as a function of encapsulated α-acetolactate decarboxylase added. (a) Diacetyl profiles of simulated accelerated fermentation involving free α-acetolactate decarboxylase. Curves from top to bottom refer to 2, 4, 6, 12 and 14 mg/L of α-acetolactate decarboxylase. (b) Simulated accelerated fermentation time as a function of α-acetolactate decarboxylase added.

With a multiplication factor of 4 for yeast-dependant reactions, α-acetolactate and diacetyl peaks occur after 1−2 days. This is consistent with the results of Kirin's team (*40*). However, profile positions are changed compared to traditional fermentation; α-acetolactate concentration is now much higher than diacetyl concentration. This fact has already been mentioned with immobilized yeast systems used in brewing (*41*). Therefore, in such a case, it is better to consider the time when α-acetolactate concentration is lower than 1.7 μM at the end of fermentation, which here is 7.5 days.

Modeling of accelerated fermentation has also been conducted with encapsulated α-acetolactate decarboxylase (see Figure 12). In presence of encapsulated α-acetolactate decarboxylase, α-acetolactate below 1.7 μM is reached after 4 days instead of 7.5, and as a result 47% of the time is gained.

Accelerated Fermentation Time versus Encapsulated α-Acetolactate Decarboxylase Concentration. The impact of encapsulated α-acetolactate decarboxylase concentration on accelerated fermentation time is shown in Figure 13. Graph 13a allowed us to build graph 13b. Here too, time to get diacetyl or α-acetolactate level lower than 1.7 μM decreases with enzyme concentration. Simulations show that with an α-acetolactate decarboxylase concentration of 14 mg/L, diacetyl never gets higher than 1.7 μM.

Conclusion

First, the use of α-acetolactate decarboxylase is a way to limit primary fermentation by accelerating the necessary decrease in diacetyl content. The aim of this study was to evaluate α-acetolactate decarboxylase encapsulation for use during beer fermentation.

The major advantage of encapsulated versus free enzyme is cost-savings because the same enzyme can be used several times as it is immobilized. From a cost point of view, if the immobilized enzyme system allows the treatment of five times the volume of beer compared to free enzyme, the cost due to enzyme is reduced by a factor of five.

The modeling approach studied here is based on synthesis and consumption of diacetyl during beer fermentation. Eight days can be gained by using encapsulated α-acetolactate decarboxylase in this way, which means a 30−35% increase in productivity. This result confirms the validity of the model and values of parameters since it is consistent with literature data.

This study shows that small capsules (1 mm diameter, 100 μm membrane thickness) make it possible to avoid mass transfer limitations usually cited as the major drawback of immobilized enzyme.

This model can be considered as a first step in a feasibility study of the use of encapsulated biocatalysts in the beverage industry. Moreover, modeling becomes an indispensable tool for decision making on further investment in the brewing industry. However, this evaluation mainly takes into consideration the kinetics. Before application of the process in breweries, it would be important to evaluate technical problem (such as mechanical resistance of capsules) and economical aspects.

Notation

P	precursor of α-acetolactate (μM)
AL	α-acetolactate (μM)
DA	diacetyl (μM)
AC	acetoin (μM)
AL_o	α-acetolactate outside the capsules (μM)
AL_i	α-acetolactate inside the capsule (μM)
AC_o	acetoin outside the capsules (μM)
AC_i	acetoin inside the capsules (μM)
TFS	total fermentable sugars (g/L)
k_0	synthesis constant for α-acetolactate (d^{-1})
k_1	synthesis constant for diacetyl (d^{-1})
k_2	α-acetolactate into acetoin specific conversion constant (L/(d mg))
k_3·[dr]	diacetyl into acetoin conversion constant (d^{-1})
aldc	α-acetolactate decarboxylase concentration (mg/L)
dr	diacetyl reductase concentration (mg/L)
A	interfacial area (m^{-1})
d	diameter of the capsules (m)
D_e	diffusion coefficient through the capsule membrane (m^2/s)
V_{m1}	maximum rate constant for α-acetolactate decarboxylase (μmol/(min mg))
K_{m1}	Michaelis constant for α-acetolactate decarboxylase (μM)
V_{m2}	maximum rate constant for diacetyl reductase (μmol/(min mg))
K_{m2}	Michaelis constant for diacetyl reductase (μM)
α	concentration of capsule in the reactor (v/v)
δ	thickness of the membrane (m)

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