Optimization of the Bioethanol Production on Sweet Cheese Whey by *Saccharomyces cerevisiae* DIV13-Z087C0VS using Response Surface Methodology (RSM)

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**Abstract**

Recently, various researches on the bioethanol production from waste have been performed for both ecological and economic reasons, mainly for its use as an alternative to petroleum based fuel. In this study, bioethanol production on cheese whey using *Saccharomyces cerevisiae* DIV 13-Z087C0VS, an Algerian yeast strain isolated from soil, was investigated. First, before the fermentation process, the lactose, main sugar in cheese whey, must be hydrolysed enzymatically with β-galactosidase to obtain fermentable sugars, mainly glucose and galactose. Second, experiment based on Central Composite Design (CCD) were conducted to study the effects of operating parameters such as temperature (25 - 35 °C), pH (3.5 - 6.5) and yeast extract supplement (0.5-1.5%) on bioethanol yield. Finally, the data obtained from the used design were subjected to the analysis of variance (ANOVA) and analysed using a second degree polynomial equation. According to the obtained results, all the studied variables had significant effect on bioethanol production with probability (P) less to 0.05. Furthermore, the results of optimization process showed that the optimum values of temperature, pH and yeast extract concentration were found to be respectively 28.38°C, 4.31 and 3.969 g/l. Under these optimized conditions, the alcoholic fermentation gave the highest bioethanol concentration of 18.53 g/l after 24 hours of incubation with the following kinetic parameters of fermentation: \( \mu_{\text{max}} = 0.328 \, \text{h}^{-1} \), \( \mu_p = 1.059 \, \text{h}^{-1} \), \( Y_{x/s} = 0.282 \, \text{g/g} \) and \( Y_{p/s} = 0.91 \, \text{g/g} \).

**Key-words:** bioethanol, cheese whey, pretreatment, *Saccharomyces cerevisiae*, optimization fermentation, Response Surface Methodology, kinetic parameters

1. **Introduction**

In recent years, several researches were investigated in the bioethanol production because it is considered as one of the promising future energy alternatives contributing to the reduction of negative environmental impacts generated by the use of fossil fuels [1]. It is widely used as partial gasoline replacements in the world and it may be produced from different substrates, including agricultural crops and food processing wastes [2]. Among these wastes, cheese whey which is known as byproduct of dairy industries. Its world production is estimated about 120.10⁶ tons per year [3]. Furthermore, in Algeria, the overall amount of whey discarded daily in the water course, is 6000 liters. A half of this amount is not valued so it constitutes a huge problem of environmental pollution with high BOD and COD which are respectively 30-50 g/l and 60-80 g/l [4]. According to the richness of cheese whey in nutrients, lactose (4.5-5% w/v), soluble proteins (0.6-0.8 % w/v), fats (0.4-0.5 % w/v) and mineral salts (8-10 % of dried extract) [5], this product is used as a medium by converting
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Lactose to ethanol using several microorganisms fermenting lactose. These microorganisms are inhibited by moderate sugars and ethanol concentrations [6]. They have also relatively slow growth and low ethanol tolerance compared to other yeast strains such as *Saccharomyces cerevisiae* which is non-fermenting lactose yeast. Therefore for this reason, lactose must be prehydrolysed enzymatically to obtain fermentable sugars (glucose and galactose) using β-galactosidase enzyme. In the present study, our aims are: i) valuation of cheese whey as a medium for production of a new metabolite, bioethanol, known as renewable energy, using an Algerian yeast strain *Saccharomyces cerevisiae* DIV13-Z087C0VS; ii) optimization of the biotechnological conditions for bioethanol production using Response Surface Methodology (RSM).

2. Materials and Methods

2.1. Yeast strain

*Saccharomyces cerevisiae* DIV13-Z087C0VS was isolated from Algerian soil and identified by physiological, biochemical and molecular methods (Laboratory of mycology, Louvain-la-Neuve University, Belgium). The stock culture was maintained in Yeast Peptone Glucose (YPG) agar medium containing (g/l): yeast extract 10, peptone 5, glucose 20 and agar 20, pH 5.0. After 48 hours of incubation, at 28 °C, the culture was stored at 4 °C.

2.2. Carbohydrates fermentation test

The ability of our yeast strain to ferment and assimilate some carbohydrates was carried out in Durham tubes containing YP broth with 2% (w/v) of each tested sugar (glucose, galactose and lactose) and in the presence of Bromocresol blue as pH indicator. The tubes were incubated at 28°C for three days and checked daily for growth development, gas production and color change of the medium.

2.3. Fermentative medium preparation

Cheese whey obtained from cheese industrial unit of Boudouaou (Boumerdes, Algeria) was deprotenized according to the method of Moulin et al.,1976 [7].

The pH of cheese whey was adjusted to 4.6 with 1N HCl and heated at 90°C for 10 min. This treatment induced the precipitation of proteins which were removed by filtration through filter paper (Whatman). The pH of the medium was adjusted to 7.0 with 1N NaOH and autoclaved at 121°C for 20 min.

2.4. Enzymatic hydrolysis

In a 250 ml Erlenmeyer flask, 1.5 mg of β-galactosidase from *Aspergillus oryzae* G 5160-500 KU Sigma (enzyme activity of 10.3 Unit /mg) was added aseptically into 100 ml of cheese whey adjusted at pH 4.5 and incubated at 28 °C for 24 h. This pretreatment assures the conversion of all quantity of lactose to monosaccharides, glucose and galactose.

2.5. Fermentation conditions

A colony unit forming of yeast pure culture cultivated on agar medium was transferred to glass tube containing 10 ml of YPG broth and incubated at 28°C for 24 hours. Five milliliters of this culture were then transferred into 10 ml of sterilized YGP medium and incubated at 28 °C in rotary shaker at 150 rpm for 18 h [8]. The active cells were centrifuged at 8000 g for 10 min and further washed with sterile distilled water to be used as inoculum. The batch fermentations were carried out in series of 250 ml Erlenmeyer flasks, containing 100 ml of pretreated and sterilized cheese whey. The inoculum (10 % v/v) was dispensed to each flask and shaken at 150 rpm. All the experiments were performed three times and the mean value was calculated.
2.6. Experimental design and statistical analysis

The optimization of the bioethanol production from cheese whey was studied using a Central Composite Design (CCD) and three factors were chosen as independent variables; temperature (X₁), pH (X₂) and yeast extract supplement (X₃). According to this CCD, 20 experiments were performed and included eight trials for factorial design, six trials for axial points and six trials for replication of central points (Table 1). The independent variables for the development of regression equation were shown to the following:

\[ x_i = \frac{X_i - X_c}{\Delta x_i} \quad i = 1, 2, 3 \]

where \( x_i \) is the coded value for the independent variable. \( X_i \) is the natural value, \( X_c \) is the natural value at the center point and \( \Delta x_i \) is the step change value.

The response surface model was fitted to the response variable, namely bioethanol amount (g/l). The second degree polynomial function is given by eq 1:

\[ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1X_2 + \beta_{13} X_1X_3 + \beta_{23} X_2X_3 \]

where \( X_1, X_2, X_3 \) represent the levels, \( Y \) is the response variable, \( \beta_0 \) is the intercept. \( \beta_1, \beta_2 \) and \( \beta_3 \) are linear coefficients. \( \beta_{11}, \beta_{22} \) and \( \beta_{33} \) are the squared coefficients. \( \beta_{12}, \beta_{23}, \beta_{23} \) are the interaction coefficients and \( X_1, X_2, X_3, X_1^2, X_2^2, X_3^2, X_1X_2, X_1X_3, X_2X_3 \) are the levels of independent variables [9].

Table 1. Experimental range and levels of the independent variables used in the CCD

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Range and levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low level star point (( \alpha = -1.68 ))</td>
</tr>
<tr>
<td>Temperature (( X_1 ))</td>
<td>21.6</td>
</tr>
<tr>
<td>pH (( X_2 ))</td>
<td>2.48</td>
</tr>
<tr>
<td>Yeast extract concentration (% w/v) (( X_3 ))</td>
<td>0.16</td>
</tr>
</tbody>
</table>

2.7. Analytical methods

Total solids, ash and fat amount were determined in cheese whey according to the methods described by AFNOR (1986) [10]. The pH value was measured at 20 °C by a pH meter (Hanna Model-pH 209, Canada). The total nitrogen and protein contents were determined by Kjeldahl method [11]. The estimation of reducing sugars (lactose) was carried out using 3,5- dinitrosalycilic acid (DNS) method described by Miller (1959) [12]. The cell growth was determined by optical density measurement at 600 nm using UV visible spectrophotometer (Varian 50 Tablet ,USA). The biomass was estimated according to the dry weight method [13]. The yeast cells were harvested by centrifugation at 7000 g for 10 min. Then, the pellet was washed with distilled water and dried over 2 hours at 105 °C until constant weight. A calibration curve between the optical density (OD) at 600 nm and dry weight was further established. Ethanol was measured directly from the samples of the fermentation using ebulliometer Dujardin Salleron (France) [14].

2.8. Statistical analysis

The data were analysed using MINITAB software 15 (trial version) to obtain a quadratic mathematical equation. The statistical analysis of regression coefficient was performed using analysis of variance (ANOVA). It included the following parameters; coefficient of
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determination $R^2$, Student test (t), Fisher test (F) and P-value. In our study, the statistical significance test level was set at 5\% ($\text{Probability (p)} < 0.05$).

3. Results and discussion

3.1. Carbohydrates fermentation

As indicated in Table 2, it appears clearly that our yeast strain is capable to ferment and assimilate both glucose and galactose in its growth. On contrary, this strain presents a negative test with lactose. El-Nemr (2001) [15] reported that \textit{Saccharomyces cerevisiae} cells are unable to ferment lactose due to the lack of lactase or $\beta$ galactosidase system.

According to Kurtzman and Fell (1998) [16], \textit{Saccharomyces cerevisiae} is one of the oldest exploited and best studied microorganisms in both old and new biotechnologies and is known to be first industrial microorganisms which readily convert sugar into alcohol and CO$_2$ in metabolic process called fermentation. However, lactose is the main sugar in cheese whey and it is no fermented by tested stain, for this reason, the lactose must be hydrolysed enzymatically into fermentescible sugars, such as glucose and galactose.

\begin{table}[h]
\centering
\caption{Carbohydrates fermentation test}
\begin{tabular}{|c|c|}
\hline
Carbohydrates & Results \\
\hline
D-Glucose & + \\
D-Galactose & + \\
Lactose & - \\
\hline
\end{tabular}
\end{table}

3.2. Chemical characterization of cheese whey

As shown in Table 3, the chemical composition of sweet cheese whey from cheese industrial unit of Boudouaou is not very different of that obtained by Sottiez (1990) [17]. However, the compound values are slightly lower or higher which can be explained by deference to the initial composition of milk used in cheese production. Moreover, we note that the lactose is the most important component of cheese whey; it represents 63 g/l. According to Lejeune and Baron (1995) [18], cheese whey is an energy substrate and is the main source of carbon and energy for the growth of several microorganisms.

\begin{table}[h]
\centering
\caption{Chemical characterization of cheese whey}
\begin{tabular}{|c|c|}
\hline
Sweet cheese whey & \\
\hline
pH & 6.2 \\
Acidity (D°) & 7.6 \\
\textbf{Composition in g/l} & \\
Total solid & 64.3 \\
Ash & 6.97 \\
Fat & 1.8 \\
\textbf{Total nitrogen} & 1.088 \\
\textbf{Protein amount} & 6.8 \\
\textbf{Reducing sugars} & 63 \\
(lactose) & \\
\hline
\end{tabular}
\end{table}

3.3. Optimization of Operating Parameters for Bio ethanol Production

Twenty experiments based on the CCD were carried out with a various combination of independents variables (temperature, pH and yeast extract supplement) and the results are presented in Table 4.
According to this study, a second order polynomial (model proposed) for bioethanol production is regressed only by considering the significant terms as shown in the following equation:

\[ Y = 17.93 - 0.60 X_1 - 0.76 X_2 - 0.56 X_3 - 1.41 X_1^2 - 1.40 X_2^2 \]

The regression coefficients, along with the corresponding t and P-values, for all the linear, quadratic and interaction effects of tested parameters are described in Table 5. A positive sign in t-value indicates a synergistic effect and a negative one represents the antagonistic effect of the tested parameters on bioethanol production. All the regression coefficients of linear and quadratic terms are statistically significant at \( p \leq 0.03 \) level, except a quadratic term for yeast extract supplement, which is not significant (\( p \leq 0.183 \)). In addition, the Table 4 shows also that the pH has a high significance (\( p \leq 0.006 \)) compared to the other parameters, the temperature and the yeast extract supplement with respectively (\( p \leq 0.020 \)) and (\( p \leq 0.029 \)). However, the results indicate that all the interactions are not significant (\( p \leq 0.445 \)).

### Table 4. Coded and uncoded full-factorial CCD for the three independent variables

<table>
<thead>
<tr>
<th>Run order</th>
<th>Coded levels</th>
<th>Real values</th>
<th>Ethanol Concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X₁</td>
<td>X₂</td>
<td>X₃</td>
</tr>
<tr>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>+1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>4</td>
<td>+1</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>5</td>
<td>-1</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>6</td>
<td>+1</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>7</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>8</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>9</td>
<td>+1.68</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>-1.68</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>+1.68</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>-1.68</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
<td>+1.68</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>-1.68</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 5. Estimated regression coefficients, t and P-values of the model

<table>
<thead>
<tr>
<th>Terms</th>
<th>Coef</th>
<th>Coef Err</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>constant</td>
<td>17.9345</td>
<td>0.3320</td>
<td>54.020</td>
<td>0.000</td>
</tr>
<tr>
<td>X₁</td>
<td>-0.6093</td>
<td>0.2203</td>
<td>-2.766</td>
<td>0.020</td>
</tr>
<tr>
<td>X₂</td>
<td>-0.7609</td>
<td>0.2203</td>
<td>-3.454</td>
<td>0.006</td>
</tr>
<tr>
<td>X₃</td>
<td>-0.5600</td>
<td>0.2203</td>
<td>-2.542</td>
<td>0.029</td>
</tr>
<tr>
<td>X₁X₁</td>
<td>-1.4101</td>
<td>0.2144</td>
<td>-6.576</td>
<td>0.000</td>
</tr>
<tr>
<td>X₁X₂</td>
<td>-1.4030</td>
<td>0.2144</td>
<td>-6.543</td>
<td>0.000</td>
</tr>
<tr>
<td>X₁X₃</td>
<td>-0.3070</td>
<td>0.2144</td>
<td>-1.432</td>
<td>0.183</td>
</tr>
<tr>
<td>X₂X₂</td>
<td>0.3675</td>
<td>0.2878</td>
<td>1.277</td>
<td>0.230</td>
</tr>
<tr>
<td>X₂X₃</td>
<td>0.1125</td>
<td>0.2878</td>
<td>0.391</td>
<td>0.704</td>
</tr>
<tr>
<td>X₃X₃</td>
<td>0.3050</td>
<td>0.2878</td>
<td>1.060</td>
<td>0.314</td>
</tr>
</tbody>
</table>

\[ R^2 = 91.47\% , R^2(adjust) = 83.80\% , S = 0.814019 , \text{Som Car-ErrPrév} = 51.4094 \]
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ANOVA of the regression coefficients for bioethanol production (Table 6) demonstrates that the model is significant due to the F-value of 11.92 and low probability P-value ($p \leq 0.0000$). Generally, the F-value with a low probability P-value indicates high significance of the regression model [19].

<table>
<thead>
<tr>
<th>Source</th>
<th>Degree of freedom (DF)</th>
<th>Sum of squares</th>
<th>Mean square (MS)</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>9</td>
<td>71.0956</td>
<td>7.8995</td>
<td>11.92</td>
<td>0.000</td>
</tr>
<tr>
<td>- Linear</td>
<td>3</td>
<td>17.2588</td>
<td>5.7529</td>
<td>8.68</td>
<td>0.004</td>
</tr>
<tr>
<td>- Squar</td>
<td>3</td>
<td>51.9109</td>
<td>17.3036</td>
<td>26.11</td>
<td>0.000</td>
</tr>
<tr>
<td>- Interaction</td>
<td>3</td>
<td>1.9259</td>
<td>0.6420</td>
<td>0.97</td>
<td>0.445</td>
</tr>
<tr>
<td>Interaction residual error</td>
<td>10</td>
<td>6.6263</td>
<td>0.6626</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Lack of fit</td>
<td>5</td>
<td>6.6263</td>
<td>1.3253</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Pure error</td>
<td>5</td>
<td>0.0000</td>
<td>0.0000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>77.7219</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The evaluation of the model is checked using regression correlation coefficient ($R^2$) which corresponds to 0.9147 (Figure 1). This value indicates that 91.47% of the variability in the response could be explained by the model with elimination of 8.53% of the total variation in bioethanol production. Chauhan and Gupta, 2004[20] have emphasized in their work, the acceptance of any model with $R^2 > 0.75$ (75%). For a good statistical model, the $R^2$ value should be in the range of 0 -1.0, and nearer to 1.0 [21].

![Figure 1. Evolution of experimental values of ethanol concentration as function as predicted values of ethanol concentration.](image)

3.4. Effect of operating parameters on bioethanol yield

Three D response surface curves are plotted to explain the effect of operating parameters (temperature, pH and yeast extract) and the optimum value of each parameter required for bioethanol production.
Figure 2. Response surface showing the effect of temperature and pH on bioethanol yield (Maintained value yeast extract 1%).

Figure 3. Response surface showing the effect of temperature and yeast extract on bioethanol yield (Maintained value pH 5.0).

Figure 4. Response surface showing the effect of pH and yeast extract on bioethanol yield (Maintained value temperature 30°C).

Figure 2 shows the response against temperature and pH while yeast extract concentration is maintained at its centre point value (1% w/v).

Hence, the bioethanol concentration increases with temperature increasing and pH in the range 25 °C to 28.38 °C and 2.48 to 4.31 respectively. However, it’s reduced for further increase in temperature and pH above 28.38°C and 4.31 respectively. Depending on the results of De Vasconcelos et al., 1998[22] and Nigam, 1999[23], this reduction is mainly due to the denaturation of yeast cells. The deleterious effect of higher temperatures on ethanol yield can be attributed to the denaturation of ribosomes, enzymes and problems associated with the fluidity of membrane [24]. In addition to the effect of high temperature, Pena et al.,
1972[25] reported that the inhibitory effect of pH (at the high level) on the ethanol yield could be due to the low ATP production during the metabolic changes in *Saccharomyces cerevisiae*.

Figure 3 reveals the effect of temperature and yeast extract supplement on bioethanol production with a fixed value of pH (pH = 5.0) while figure 4, represents effect of pH and yeast extract supplement on bio ethanol production with a maintained value of temperature (30°C). It can be observed that the increase of yeast extract concentration together with increase of temperature and pH limits the bioethanol production. Chan-u-tit et al., 2013[26] indicated in their works that the increasing in yeast extract concentration in the medium did not promote ethanol production. Therefore, Bafrcová et al., 1999 [27] reported that excess nitrogen did not lead to an increase in ethanol production rate and a reduction of fermentation time. In the present study, it clearly appears that with lowest value of yeast extract supplement (0.4 % w/v) and closely in the centre point values of temperature (30°C) and pH (5.0), the bioethanol production presents a best fermentation performance. Generally, nitrogen sources are added to enhance yeast growth and ethanol production and, specially, yeast extract is proven to be very efficient for increasing fermentation rate because it primarily consists of amino acids, peptides, nucleotides and other soluble components of yeast cells [28].

The RSM shows that the optimized conditions for bioethanol production on pretreated cheese whey using *Saccharomyces cerevisiae* DIV13-Z087C0VS are found to be temperature of 28.38°C, pH = 4.31 and yeast extract supplement of 0.4 % (w/v) with a predicted value of bioethanol concentration of 18.53 g/l.

The optimum of temperature (28.38°C) confirms the result of Yinling et al., 2011[29] who found a value of 27 °C for producing a bioethanol on a very high gravity Cassava mash using *Saccharomyces cerevisiae*. However, Fakruddin et al., 2012[30] noted 30°C as optimum temperature for *Saccharomyces cerevisiae* IFST-07201 using molass as medium while Yah et al., 2010 [31] reported 25°C for optimum ethanol production by *Saccharomyces cerevisiae*. Gonzalez et al., 1996 [32] recommended a range of temperature between 18 and 32 °C for bio ethanol production by *Saccharomyces cerevisiae*.

Concerning the pH optimum (4.31), it is clearly observed that our result is in accordance with DeVascocelos et al., 1998 [22] and Nigam, 1999[23] who signaled a maximum productivity of bio ethanol by *Saccharomyces cerevisiae* at pH of 4.2 to 4.5. Chaing et al., 1981 [33]. Narendranath and Power, 2005 [34] have also optimum pH in range 4.0 to 6.0. It is commonly known that *Saccharomyces cerevisiae* is an acidophilic organism [35].

The supplementation of cheese whey with 0.4 % of yeast extract (3.969 g/l) has significantly improved the bioethanol production yield, this value is considered as an optimum operating parameter. The best bioethanol production was found by Deesuth et al., 2012 [36] to be in range 3.5 to 7 g/l whereas Nuanpeng et al., 2011 [37] reported that the maximum efficiency was obtained when 9 g/l of yeast extract was supplemented to the fermentation medium.

### 3.5. Production of bioethanol in optimized conditions

The batch fermentation on prehydrolysed cheese whey with initial sugar concentration of 39.39 g/l using *Saccharomyces cerevisiae* DIV13-Z087C0VS provides a maximum bioethanol concentration of 17.06 g/l and a biomass concentration of 6.3 g/l after 24 hours of incubation consuming almost 76 % of the total sugar (figure 5).

The experimental data of biomass concentration as a function of the incubation time (figure 6), indicates that the experimental data are fitted well and follow the logistic model according to the following equation:

\[
Y = A_1 + \frac{(A_1 - A_2)}{[1 + (t / t_0 )^B ]}
\]

were : \( A_1 = 0.358 \), \( A_2 = 7.602 \), \( t_0 = 10.237 \), \( B = 1.977 \), \( R^2 = 0.977 \)
Figure 5. Evolution of ethanol concentration, biomass concentration and total reducing sugar as a function of time at optimized conditions (□: biomass (g/l), ◊: Ethanol concentration (g/l), △: Total reducing sugar (g/l)).

Figure 6. Logistic kinetic model fitted with experimental data.

Figure 7. Evolution of specific bioethanol production rate as function of specific biomass formation rate.

In addition, Luedicking and Piret model was evaluated for bioethanol production. The figure 7 represents variation in specific production rate as a function of the specific growth. It shows that the bioethanol production by tested yeast strain is “growth associated” with correlation coefficient $R^2 = 0.9994$, $\alpha = 3.2096$ g/g and $\beta = 0.0071$ g/g.h ($\alpha$ and $\beta$ are empirical constants that vary with fermentation conditions).
Similar results were found by Manikandan et Viruthagiri, 2010 [38] who produced a bioethanol by *Saccharomyces cerevisiae* MTCC 463 on corn flower where $\alpha = 2.17$ g of product / g of biomass and $\beta = 0.062$ g / g.h. Elumalai and Thangavelu, 2010 [39], Kostov et al., 2012 [40] and Jiménez-Islas et al., 2014 [41] have also evaluated Luediking and Piret model for bioethanol production using *Saccharomyces cerevisiae* and proposed a growth associated production.

In contrary, Ahmad et al., 2011 [42] noted that the ethanol production is non associated growth process with aeration of 0.075 w and agitation speed of 75 rpm. According to Jiménez-Islas et al., 2014 [41], this discrepancy can be explained by the fact that when oxygen is absent, *Saccharomyces cerevisiae* produces ethanol in order to re-oxidize NADH, H$^+$ to NAD$^+$ and when oxygen is present, it is the final electron acceptor.

The kinetic parameters of growth and bioethanol production under optimized conditions are summarized in table 7. It shows that the maximum growth rate is 0.325 h$^{-1}$ with biomass yield from sugar of 0.282 g/g while the maximum ethanol production yield is 1.059 h$^{-1}$ and $Y_{p/s} = 0.91$ g / g.

| Biomass formation rate $r_x$ (g/l.h) | 0.350 |
| Ethanol formation rate $r_p$ (g/l.h) | 1.13 |
| Sugar consumption rate $r_s$ (g/l.h) | 1.24 |
| Specific biomass formation rate $\mu_x_{max}$ (h$^{-1}$) | 0.328 |
| Specific ethanol production rate $\mu_p_{max}$ (h$^{-1}$) | 1.059 |
| Biomass yield from sugar $Y_{x/s}$ (g/g) | 0.282 |
| Ethanol yield from sugar $Y_{p/s}$ (g/g) | 0.91 |
| $\alpha$ | 3.2096 |
| $\beta$ | 0.0071 |

### 4. Conclusions

A Central Composite Design (CCD) has been used in the present study for the optimization of operating conditions. It establishes an efficient second order polynomial model to describe the studied biotechnological conditions. By solving the regression equation, the optimum values of temperature, pH and yeast supplement are found to be respectively 28.38°C, 4.31 and 0.4 %. A maximum ethanol concentration of 18.53 g/l is obtained in optimized conditions. In addition, logistic model and Luèdiking-Piret model represent approximately the experimental data of both growth and bioethanol production kinetics. Luèdiking and Piret model confirms that the bioethanol production on prehydrolyzed cheese whey using *Saccharomyces cerevisiae* DIV 13-Z087C0VS is a growth associated with $\alpha = 3.2096$ g of bioethanol / g of biomass and $\beta = 0.0071$ g of bioethanol / g of biomass.

### References

Optimization of the Bioethanol Production on Sweet Cheese Whey by *Saccharomyces cerevisiae*
DIV13-Z087C0VS using Response Surface Methodology (RSM)


