Studies on optimization conditions for alcoholic fermentation process of delta beet molasses

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Abstract:

A mixture sample of beet molasses from production line I of DELTA sugar factory at period from 9/2 to 12/2 during season 2010 was used as a good fermentation medium in this study. Sugar levels of molasses were adjusted to different concentrations (5, 10, 15, 20, 25 and 30%) using distilled water. The results appeared that the highest level of ethanol production was 9.2% (equal 90% of theoretical values). This level was recorded when used molasses with 20% sugar and grown for 72 hours. To know the optimum temperature for ethanol fermentation, the fermentation process was kept at 25, 30, 35 and 40°C. Ethanol yield reached to maximum and turned out to be 9.2% after 48 hours at 30°C. Also, effect of pH on ethanol production level was examined and the results appeared that the maximum ethanol level was obtained at pH 5.

Introduction:

During industrial scale of ethanol fermentation yeast- encounters a multitude of stress factors that impose constrains on yeast growth as well as fermentative metabolism. These stresses include high sugar concentration, elevated temperature, high ethanol concentration and low external pH. So, this investigation was designed to study the effect of each of sugar concentration, temperature and pH on the concentration of ethanol production using DELTA beet molasses.

Use of concentrated sugar substrate is one of the ways to obtain high ethanol yield during fermentation. However, Jones et al. (1994) found that the high sugar concentrations are inhibitory to fermentation due to osmotic stress. Borzani et al. (1993) studied fermentation with various initial concentrations of sugar and demonstrated the logarithmic relationship between time of fermentation and initial concentrations of sugar. Bertolini et al. (1991) isolated yeast strains had the ability to ferment up to 30% sucrose efficiently from sample collected from Brazilian alcohol factories. They recorded that the efficiency of selected strains varied from 89% to 92% depending upon the utilization of total
sugar available in the medium with maximum amount of 19.7% (v/v) ethanol accumulated. A repeated batch fermentation system was used to produce ethanol using an osmotolerant \textit{S. cerevisiae} (US3) immobilized on calcium alginate (Sree et al., 2000). Their fermentation was carried out with initial concentration of 150, 200, 250 g glucose per liter at 30°C and the maxima amounts of ethanol produced were 72.5, 93 and 83 g ethanol per liter at 30°C after 48h.

The fermentation process is always accompanied with evolution of heat that raises the temperature of the fermentor. As a result it becomes necessary to cool the large fermentors in the distilleries. This necessity often becomes a major operation and a cost factor in the production of ethanol. Temperature exerts a profound effect on growth, metabolism and survival of the fermenting organism. Fermentation in industries is usually carried out at ambient temperature of 25-35°C but temperature exceeds 40°C during fermentation especially in northern regions which decreases the cell viability and productivity. Maintenance of high cell viability is a major characteristic of fermentation to get high ethanol yield.

Fermentation at 35-40°C or above has advantages such as ethanol recovery and significant savings on operational costs of refrigeration control in distilleries for alcohol production. Therefore many studies have been carried out for development of yeast to ferment at high temperature of up to 40-45°C. Laluce et al. (1993) studied the effects of temperature on fermentation capacity of three yeast strains 19G, 78I and baker’s yeast in complete medium and sugarcane juice broth containing 15% total sugar. They reported that a complete conversion of total sugar to ethanol was observed after 12 hrs of fermentation at 39-40°C. They also observed that above 40°C, a strong inhibitory effect on ethanol production was occurred.

Singh et al. (1998) studied the ethanol production at elevated temperatures. They isolated a number of strains of \textit{Kluyveromyces marxauus} var. marxianses capable of growth at high temperatures coupled with production of high alcohol concentrations by fermentation of glucose and molasses.

Morimura et al. (1997) made an attempt to improve the salt tolerance of the thermotolerant flocculating yeast \textit{Saccharomyces cerevisiae} strain KF-7 by maintaining a high concentration of KCl in the molasses medium. Among selected strains, K211 had the highest cell viability and ethanol productivity in a molasses medium containing 25% (w/v) total sugar at 35°C.

Yadav et al. (1997) found an increase in alcohol concentration productivity as well as efficiency with an increase in pH from 4.0-5.0 and found that the optimum pH range for \textit{S. cerevisiae} to be between pH 4.5 - 5.0. Early, Prescott and Dunn (1959) reported that the yield of 90% ethanol or more of the theoretical values were obtained under the optimum fermentation condition. They
also reported that the efficiency of ethanol fermentation depends on the pH and they suggested that a pH of 4 to 4.5 favour the yeast growth, but is sufficiently low to inhibit the development of many type of bacteria.

Russell (2003) recorded that yeast prefers an acid pH and its optimum pH is 5.0 – 5.2. Narendranath and Power (2005) found that the optimum pH for yeast growth and ethanol production by S. cerevisiae was pH 4.9. Limtong et al. (2007) examined the ethanol production from sugar cane juice with 22% sugar and found that the highest ethanol concentration was obtained when the fermentation carried out at pH 5.0.

Material and methods

Molasses Samplers

A mixture sample of beet molasses from production line I of DELTA sugar factory at period from 9/2 to 12/2 during season 2010 was collected and used as a good fermentation medium in this study.

Yeast Strain

Yeast strain was Saccharomyces cerevisiae AU 71 (thermotolerant and high ethanol producing strain) which kindly obtained from Industrial Microbiological Laboratory, Botany Department, Faculty of Science, Assiut University.

Optimization of fermentation process

The collected mixture sample of beet molasses was supplemented with 0.1% KH$_2$PO$_4$ & 0.2% (NH$_4$)$_2$SO$_4$ and used in the following experiments for optimized the ethanol production conditions from beet molasses.

a) Effect of sugar concentration

To study the effect of sugar concentration on ethanol production by S.cerevisiae, the production media was prepared by diluting selected molasses to sugar concentration of 5, 10, 15, 20, 25, 30 percent with distilled water. The diluted samples of beet molasses were heated at 95°C for 20 min, then cooled and filtered through ordinary filter paper to remove suspended particles. The prepared inoculum of selected yeast strain was added at the rate of 10 percent to 100 ml of the medium in 200 ml sterilized bottles. Prepared molasses media were adjusted to pH 5.0. Fermentation carried out at 120 rpm and 30°C for different incubation periods up to 72 hours under anaerobic condition. Samples were withdrawn after every 12 hours interval and determined the dry yeast weight as well as ethanol content in the fermentation medium. The percentage of ethanol was calculated. The initial sugar concentration that was efficiently utilized by the yeast for ethanol production was selected for further experiments.
b) Effect of temperature

To optimize the fermentation temperature, fermentation was carried out at 25, 30, 35 and 40°C. Molasses diluted to 20% sugars. Prepared molasses were heated at 95°C for 20 min, thin cooled and filtrated. The pH was adjusted to 5.0. Fermentation was carried out in 200ml sterilized bottles which had 100 ml of prepared molasses medium and inoculated with prepared inoculum of selected yeast at rate of 10%. Cultures were incubated at 120 rpm for 48 hours. The periodic samples were analyzed for dry yeast weight and ethanol content.

c) Effect of pH

Different levels of pH (5.0, 6.0, 7.0 and 8.0) were tested for fermentation using molasses with 20% sugar concentration. Prepared molasses were heated at 95°C for 20 min, then cooled and filtered before adjusted the pH. Fermentation was carried out in 200ml sterilized bottles (had 100 ml of prepared molasses medium and inoculated with prepared inoculum of selected yeast at rate of 10%) at 30°C and 120 rpm for 48 hours. Periodic samples (at each 12 hours) were analyzed for dry yeast weight and ethanol content.

**Analytical concepts**

a) **Physicochemical analysis:** Each of brix%, specific gravity, viscosity, colour brix %, pH, total sugar%, non-fermentable sugar%, fermentable sugar% and sulfated ash% were analyzed using analytical methods of International Commission for Uniform Methods of Sugar Analysis (ICUMSA) at Delta sugar factory, quality control department as routine and classical quality control analysis.

b) **Detection of Ethanol:** Ethanol contents were estimated by dichromate method described by Zohri and Eman Mostafa (2000).

c) **Detection of yeast cells viability:** Direct viable cell count was carried out using a hemacytometer after staining with 0.1% methylene blue (Borzani and Vario, 1958). Purity of yeast cells was examined by light microscope.

d) **Determination of yeast growth:** Dry biomass was measured by filtered the yeast biomass using known weight filter paper, washed three times with distilled water and dried for 24 hours at 85°C. Dry yeast biomass was calculated from re-weight the dried filter paper plus biomass.

e) **Detection of pH value:** The pH measures were performed directly using a pH meter (Microprocessor pH – mV meter pH 526).

f) **Determination of sugar levels:** sugars were determined by the method described by the European Community (2000) as official methods for detection of sugar in wines.
Results and Discussion

Average of Physicochemical analysis of Delta beet molasses sample:

A mixture sample of beet molasses from production line I of DELTA sugar factory at period from 9/2 to 12/2 during season 2010 was used as a good fermentation medium in this study. Physicochemical analysis of this sample was achieved and the results recorded in Table (1). The result appeared the high level of fermentable sugar and their suitability for ethanol fermentation.

Table (1): Average of Physicochemical analysis of Delta beet molasses collected from the Line (I) at period from 9/2 to 12/2 during campaign 2010.

<table>
<thead>
<tr>
<th>line I</th>
<th>Brix%</th>
<th>specific gravity</th>
<th>Viscosity (cp)</th>
<th>Color % Brix</th>
<th>pH</th>
<th>Total sugar%</th>
<th>Nonfermentable sugar %</th>
<th>Fermentable sugar %</th>
<th>sulfated ash %</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/2 to 12/2</td>
<td>80.0</td>
<td>1.4142</td>
<td>1265.0</td>
<td>31320</td>
<td>9.5</td>
<td>53.7</td>
<td>0.90</td>
<td>52.80</td>
<td>13.03</td>
</tr>
</tbody>
</table>

Optimization studies on fermentation of molasses:

a) Effect of sugar concentration

Sugar levels of molasses were adjusted to different concentrations (5, 10, 15, 20, 25 and 30%) using distilled water. Fermentation process was achieved in 200 ml bottles at 30°C for 72 hours and incubated at 120 rpm. The results obtained were recorded in (Table, 2) and illustrated in (Figure,1). The results appeared that the highest level of dry yeast weight was 3.09 g/l and recorded when used molasses with 20% sugar and grown for 72 hours (Table, 2). The highest level of ethanol production was 9.2% (equal 90% of theoretical values) and recorded after 48 hours when used molasses with 20% sugar (Table, 2 & Figure, 1).

This result is harmony with those recorded by Maysa Ali (2010). She examined the effect of sugar concentration (12.5, 15, 17.5, 20, 22.5, 25, 30 and 35%) on ethanol production by five yeast strains and reported that the maximum ethanol level by *Kluyveromyces marxianus* GU133329 was 9.55% (equal 93.63% of the theoretical value) at 20% sugar concentration. Zohri and Eman Mostafa (2000) studied the effect of different sugar concentrations (13.5, 18 and 22.5%) in the date juice on the fermentation efficiency of two yeast strains and found that
the date juice with 18% sugars was the more suitable and economic concentration. Gough et al. (1998) found that a sugar concentration more than 23% in molasses had a harmful effect on ethanol production.

As general, increasing sugar concentration (at 25 & 30%) lead to increase the viscosity in fermentation medium and this had an inhibitory effect on yeast growth and their capability to ethanol production. Similar results were recorded previously in the fermentation medium with high gravity worts (Pratt- Marshall et al., 2002, 2003). They observed that fermentation of high gravity worts have a negative effect upon the yeast performance due to the elevated osmotic pressure. Reddy and Reddy (2006) reported that the increasing in sugar concentration will be decrease in sugar utilization, which results in reduction of the total ethanol production. This reduction could be due to several reasons including the production of other compounds like glycerol or acetic acid. Also, the intra-cellular ethanol (which may be increased by increasing ethanol production at high sugar concentration) exerts high toxicity on yeast and the nutrient may be deficiency (Sols et al., 1971).

Table (2): Effects of sugar concentrations on ethanol production from beet molasses using S.cerevisiae AU71.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Sugar concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry yeast weight (g/l)</td>
</tr>
<tr>
<td></td>
<td>5%</td>
</tr>
<tr>
<td>0</td>
<td>0.08</td>
</tr>
<tr>
<td>12</td>
<td>0.75</td>
</tr>
<tr>
<td>24</td>
<td>1.22</td>
</tr>
<tr>
<td>36</td>
<td>2.2</td>
</tr>
<tr>
<td>48</td>
<td>2.54</td>
</tr>
<tr>
<td>72</td>
<td>2.86</td>
</tr>
</tbody>
</table>
Figure (1): Effect of sugar concentrations in molasses medium on dry yeast weight and ethanol production by *S. cerevisiae* AU 71 grown at 30°C for 72 hours on 120 rpm.

**b) Effect of temperature**

Temperature is one of the major constraints that determine the ethanol production. To know the optimum temperature for ethanol fermentation, the fermentation process was kept at 25, 30, 35 and 40°C. The molasses were diluted up to 20% sugars and fermentation was carried out in 200ml bottles, which incubated at 120 rpm for 48 hours. Two parameters were simultaneously studied, the growth of *S. cerevisiae* AU 71 and the ethanol yield. Samples were withdrawn every 12 hours and the fermentation was carried out for 48 hours.

As shown in Table (3) and Figure (2) at 30°C, ethanol yield reached to maximum and turned out to be 9.2% after 48 hours. However increasing the temperature beyond 30°C, the growth as well as concentration of alcohol decreased. This decrease was pronounced at 40°C. Similar results were recorded by Maysa Ali (2010). She reported that the highest ethanol levels by two *Saccharomyces cerevisiae* strains were recorded at 30°C. Reddy and Reddy (2006) recommended that the fermentation temperature for ethanol production up to 30°C should considered.
Table (3): Effects of temperatures on ethanol production from beet molasses using *S.cerevisiae* AU 71.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Temperature</th>
<th>Dry yeast weight (g/l)</th>
<th>Alcohol (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25°C</td>
<td>30°C</td>
<td>35°C</td>
</tr>
<tr>
<td>0</td>
<td>0.23</td>
<td>0.25</td>
<td>0.24</td>
</tr>
<tr>
<td>12</td>
<td>0.48</td>
<td>0.74</td>
<td>0.56</td>
</tr>
<tr>
<td>24</td>
<td>0.98</td>
<td>1.45</td>
<td>1.23</td>
</tr>
<tr>
<td>36</td>
<td>1.32</td>
<td>1.89</td>
<td>1.56</td>
</tr>
<tr>
<td>48</td>
<td>1.86</td>
<td>2.83</td>
<td>1.9</td>
</tr>
</tbody>
</table>

**Figure (2):** Effect of incubation temperature on dry weight and ethanol production by *S.cerevisiae* AU 71 grown on beet molasses with 20% sugar concentration for 48 hours on 120 rpm in molasses medium.
c) Effect of pH

Initial sugar concentration in molasses medium of 20% and optimum temperature of 30°C was selected for further studies and subjected to pH treatments 5, 6, 7 and 8. The results are shown in Table (4) and illustrated in Figure (3). At pH 6, 7 & 8, fermentation took place but it gave low ethanol content. Best results were obtained at pH 5 where maximum ethanol production was noticed. Yadav et al. (1997) found an increase in alcohol concentration productivity as well as efficiency with an increase in pH from 4.0-5.0 and found that the optimum pH range for *S. cerevisiae* to be between pH 4.5 - 5.0.

Early, Prescott and Dunn (1959) reported that the yield of 90% ethanol or more of the theoretical values were obtained under the optimum fermentation condition. They also reported that the efficiency of ethanol fermentation depends on the pH or mash and they suggested that a pH of 4 to 4.5 favour the yeast growth, but is sufficiently low to inhibit the development of many type of bacteria.

Russell (2003) recorded that yeast prefers an acid pH and its optimum pH is 5.0 – 5.2. Narendranath and Power (2005) found that the optimum pH for yeast growth and ethanol production by *S. cerevisiae* was pH 4.9. Limtong et al. (2007) examined the ethanol production from sugar cane juice with 22% sugar and found that the highest ethanol concentration was obtained when the fermentation carried out at pH 5.0.

Table (4) : Effect of pH on ethanol production from beet molasses using *S. cerevisiae* AU 71.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Dry yeast weight (g/l)</th>
<th>Alcohol %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 5</td>
<td>pH 6</td>
</tr>
<tr>
<td>0</td>
<td>0.24</td>
<td>0.22</td>
</tr>
<tr>
<td>12</td>
<td>0.88</td>
<td>0.45</td>
</tr>
<tr>
<td>24</td>
<td>1.58</td>
<td>0.99</td>
</tr>
<tr>
<td>36</td>
<td>2.2</td>
<td>1.34</td>
</tr>
<tr>
<td>48</td>
<td>2.85</td>
<td>1.55</td>
</tr>
</tbody>
</table>
Figure (3): Effect of pH on dry yeast weight and ethanol production by S.cerevisiae AU 71 grown on beet molasses at 20% sugar concentration and 30°C temperature for 48 hours on 120 rpm.

References


Laluce C, Abud C J, Greenhalf W and Sanches Peres M F (1993):


