Effect of Agitational Intensity on Ethanol Production by Eco-molasses an Industrial Waste Through Yeast Sachharomyces Cerevisiae at Digitally Controlled Pilot Scale Bioreactor

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Abstract: Industrial waste Eco-molasses a richest in fermentation sources was used in pilot scale digitally controlled production of ethanol by a thermotolerant yeast specie Sachharomyces cerevisiae at elevated temperature. Effects of agitational intensity on the production of ethanol were studied during this operation through a digitally controlled Microprocessor. Ethanol production, cell mass and substrate consumption rates were enhanced manifold. It was determined that 300 rpm is an optimized agitational intensity and produce, ethanol 74.%, maximum cell growth of 9.3 g/l and 98 % of substrate was consumed. Kinetic parameters for the study and time course study for the bioreactor is also presented in the paper, which shows a better results at optimized agitational intensity and the values are comparable with the international work and is more economic.

Key words: Ethanol, Fermentation, Sachharomyces cerevisiae, Agitational intensity.

INTRODUCTION

Industrial waste, eco-molasses, a richest in sources of sugars, carbon, B-vitamins, iron, calcium, sodium and potassium was used in pilot scale digitally controlled production of ethanol by a thermotolerant yeast specie Sachharomyces cerevisiae at elevated temperature. Physico-chemical and environmental factors such as inoculum type, moisture and water activity, pH, temperature, substrate, particle size, aeration and agitation, nutritional factors, and oxygen and carbon dioxide affecting fermentation (Krishna, 2005). Mechanical mixing is that vital role in bioreactor studies. Studies, revealed those different bioreactor configurations such as and mechanical mixing. An advanced research made by other workers, such as Ahmad et al. (1994) and Tang et al. (2010) has found an enhanced traditional oxygen transfer rate as the speed of agitator raised (from 300-600 rpm) in fermentation processes (Ahmad et al., 1994; Tang et al., 2010). Greater agitation produces more dispersion hence the greater mass transfer rate Shah et al., (2009). Kaster et al. (1990) found that more dispersion could be created in low agitation if bubble dispersion is utilized for the purpose. If smaller sized bubbles incorporated then it permits more oxygen and consumes more time to dissolve Kaster et al. (1990).

MATERIALS AND METHODS

Centers:
This research work was conducted in collaboration with Mehran University of Engineering and Technology Jamshoro and National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan an institute under the administration and management of Pakistan Atomic Energy Commission (PAEC), Islamabad.

Yeast strain of S. cerevisiae:
Yeast strain of S. cerevisiae SAF (France) was purchased from a local market and grown at the most popular yeast medium at minimum composition of the constituents as shown in Tables 3.1 and 3.2 as used by Rajoka et al. (2005). Then the culture was further stabilized (through mutagenesis) for higher working temperature and catabolite repression resistant at the same time to make it thermotolerant with retention of

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hyper-production of ethanol power and used for enhanced ethanol production.

**Fermentation:**

Fermentation tanks were made up of stainless steel, having working volume of 150 liters. All experiments were performed in batch fermentation. The mutant strain was grown at 43-45 °C. The temperature was controlled by cooling water passing around the mash through the jacketed vessel. Molasses of an optimized brix / concentrations were used. Level of the fermenter was raised during agitation so one third volume of vessel was kept void in each study. This circulation ended after 28 h, by continuous adding of silicon oil as an antifoaming agent. Fermented mash was sampled at every four hour. A Brix hydrometer utilized for checking the specific and was confirmed on HPLC. Ethanol (already separated through extractor at laboratory scale) was known through HPLC. When molasses was used as a carbon source, almost 98 100 % total sugars (TS) were consumed. Peaks of ethanol were observed after a retention time of 19.30 to 19.36 minutes as mentioned in the Figure 1

![Fig. 1: HPLC Chromatogram of fermented molasses containing 15 % TS](image)

**Kinetic parameters:**

Kinetic parameters were calculated as mentioned in previous study Aiba, *et al.*, (1973). Empirical approach of the Arhenius was used to describe the relationship of temperature and product formation. Following formulae were used for the parameters:

\[
Y_{p/x} \quad \text{[Product yield coefficient with respect to cell mass]} = \frac{dP}{dx} \\
Y_{p/s} \quad \text{[Product yield coefficient with respect to substrate]} = \frac{dP}{ds} \\
q_p \quad \text{[Product yield coefficient with respect to substrate]} = \mu \cdot Y_{p/x} \\
q_s \quad \text{[Substrate utilization]} = \frac{\mu \cdot x}{Y_{x/s}}
\]

**RESULTS AND DISCUSSION**

Time course study was carried out at 150 liter fermenter for 28 h to observe ethanol production, substrate consumption and cell mass and is shown in the figures 1 to 8 and the tables 1 to 14. At the start of the fermentation for 4 h, the cell mass showed the lag phase and then the log phase started and by reaching at the peak value of 9.0 g/l, the system kept a steady state condition up to 28 h. At the larger scale like pilot size it is vital to supply Oxygen with the help of a mechanical set up known as agitator to get the air compressed. Further studies are required to know the effect of oxygen on the material present in an anaerobic reactor. The rate of oxygen at which it is being transferred and the agitational intensity is directly proportional to each other.

Production of ethyl alcohol at larger scale is crucial for any study related to fermentation that is aimed at the hyper production. Production of ethyl alcohol the yeast *S. cerevisiae* is an anaerobic process but it
requires sufficient mass of Dissolved Oxygen (D.O.) in the fermentation medium for ethyl alcohol production. Under the aeration rate of 1.0 vvm (optimized), for initial 8 h, followed by 0.25 vvm for next 20 h fermentation was optimum in the 150-litre bioreactor. Ethyl alcohol production was associated with yeast growth for 8 h and then was not associated with growth for the next 20 h (Fig 1 to 3).

In the process of fermentation, oxygen transfer is possible through the bubble as the liquid transfers in a gas. Ultimately the gas is shifted to the microorganism. This research for the oxygen shifting from a liquid to gas and gas to liquid is very necessary and it is done by air flow rate and agitation. They have a pivotal role for mass transfer of cell mass, substrate, product and temperature. Various rates of agitation were observed in the bioreactor of 150 liter volume at the optimized conditions of temperature, aeration rate, pH, Nitrogen and Carbon sources and substrate consumption in earlier experience, ranging from 200 rpm to 500 rpm (Table 3 and Figures 1 to 3) but the most effective was the rate of 300 rpm (7.4 % ethanol production, 97 % sugar utilization and 9.6 g/l cell mass production) Shah S.F.A. (2010). Where as Oniscu et al (2002) observed the rates at higher values upto 700 rpm when it was experienced for the bioreactors of smaller volumes of 5 liters Oniscu et al., (2002).

A relationship of oxygen transfer and the rate of agitational intensity in a bioreactor during the process of fermentation was established by Aldiguier et al (2004). He has proved that when an the agitational intensity of 300 to 600 will give a rise to oxygen transfer rate from 8.94 to 38.63 mmol l⁻¹·h⁻¹. He has further found that of the rate of transfer of air is enhanced from 0.21 l min⁻¹ to 1.05 l min⁻¹, the oxygen transfer rate increased from 5.7 mmol l⁻¹·h⁻¹ to 20.5 mmol l⁻¹·h⁻¹. This is done in all sorts of fermentation processes that when a rate of gas transfer occurs, the oxygen transfer increases this is very classical, higher productive and supportive one Aldiguier et al., (2004).

Table 1: Kinetic parameters for the substrate consumption, and ethyl alcohol production in a Bioreactor of 150 liter working volume at varying values of agitational intensity.

<table>
<thead>
<tr>
<th>Agitational intensity</th>
<th>µ</th>
<th>Qs</th>
<th>Qc</th>
<th>Qp</th>
<th>YX/S</th>
<th>YP/X</th>
<th>YP/S</th>
<th>qL</th>
<th>qL</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 rpm</td>
<td>0.22</td>
<td>0.27</td>
<td>6.04</td>
<td>1.7</td>
<td>0.04</td>
<td>6.21</td>
<td>0.28</td>
<td>1.37</td>
<td>5.5</td>
</tr>
<tr>
<td>300 rpm</td>
<td>0.29</td>
<td>0.40</td>
<td>6.12</td>
<td>2.54</td>
<td>0.06</td>
<td>7.71</td>
<td>0.50</td>
<td>2.2</td>
<td>4.8</td>
</tr>
<tr>
<td>400 rpm</td>
<td>0.24</td>
<td>0.34</td>
<td>6.00</td>
<td>2.79</td>
<td>0.057</td>
<td>8.3</td>
<td>0.46</td>
<td>1.2</td>
<td>4.2</td>
</tr>
<tr>
<td>450 rpm</td>
<td>0.20</td>
<td>0.30</td>
<td>4.7</td>
<td>2.16</td>
<td>0.05</td>
<td>6.58</td>
<td>0.35</td>
<td>1.22</td>
<td>4</td>
</tr>
<tr>
<td>500 rpm</td>
<td>0.17</td>
<td>0.24</td>
<td>4.1</td>
<td>1.22</td>
<td>0.037</td>
<td>5.97</td>
<td>0.31</td>
<td>1.08</td>
<td>3.6</td>
</tr>
</tbody>
</table>

The data given in the table is an average of the tow readings. The errors between values were small there fore it is not shown in the data.

Fig. 1: Effect of agitational intensity on ethyl alcohol from Wild and Mutated organisms of Sachharomyces cerevisiae. The data given in the figure is an average of the two readings.
Fig. 2: Effect of agitational intensity on Cell growth of Wild and Mutated strains of *Saccharomyces cerevisiae*. The data given in the figure is an average of the two readings.

Fig. 3: Effect of agitational intensity on substrate consumption from wild and mutated strains of *Saccharomyces cerevisiae*. The data given in the figure is an average of the two readings.

Q_p was 2.4, 3.38 and 4.5 g/l.h in S. Flask, 23 l and 150 l fermentation volume with sugar uptake rate of 3.8, 4.5, and 6.25 g/l.h respectively. Thermotolerant *S. cerevisiae* during its growth in optimized fermentation medium, containing molasses in 150 liter fermenter studies indicated that molasses supported 1.55-fold higher (Table 4.21)

Table 2: Dependence of ethanol production on culturing condition namely 23 liter, 150 liter fermenter and shake flask (S.flask) cultures: Kinetic parameters for substrate consumption (molasses) and ethanol formation by *S. cerevisiae* mutant derivative.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>23 liter</th>
<th>150 liter</th>
<th>S.flask</th>
<th>F-value</th>
<th>p-value at p≤0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>µ (h⁻¹)</td>
<td>0.34b</td>
<td>0.35a</td>
<td>0.23c</td>
<td>441</td>
<td>0.000</td>
</tr>
<tr>
<td>Q_S (g/l h)</td>
<td>4.5b</td>
<td>6.25a</td>
<td>3.8c</td>
<td>204.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Y_S/S (g/g)</td>
<td>0.06b</td>
<td>0.066a</td>
<td>0.045c</td>
<td>263.3</td>
<td>0.0000</td>
</tr>
<tr>
<td>q_e (g/g h)</td>
<td>5.7a</td>
<td>5.5a</td>
<td>5.6ª</td>
<td>0.120</td>
<td>0.459</td>
</tr>
</tbody>
</table>
Table 2: Continue

<table>
<thead>
<tr>
<th>Product formation parameters</th>
<th>Q_p (g/l h)</th>
<th>Y_P/S (g/g)</th>
<th>Y_P/X (g/g)</th>
<th>q_P (g/g h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.38b</td>
<td>0.49b</td>
<td>7.9b</td>
<td>2.7b</td>
</tr>
<tr>
<td></td>
<td>4.5a</td>
<td>0.50a</td>
<td>8.30a</td>
<td>3.0a</td>
</tr>
<tr>
<td></td>
<td>2.4 c</td>
<td>0.45c</td>
<td>8.10c</td>
<td>1.7c</td>
</tr>
<tr>
<td></td>
<td>331.24</td>
<td>63.0</td>
<td>183.0</td>
<td>139.0</td>
</tr>
</tbody>
</table>

Lane 1: Each columns 1, 2, and 3 Duncan multiple range test applying ANNOVAII in MstatC software (n=2).

Furthermore, the substrate uptake rate was improved 1.39 and 1.64-fold over that in 23 liter and shake flask cultures respectively. The influence of treatments on all fermentation attributes of ethanol production was highly significant except for (Mizuno et al. 2006; Phisalaphong et al. 2006; Rashad 2003; Sridhar et al. 2000; Banat et al. 1998) *K. marxianus* and its mutant. Ethanol volumetric productivity, ethanol yield and theoretical yield on optimized medium in 150 liter fermenter were 4.5 g/lh, 0.50 and 98 % using 150 g TS/liter in molasses. Previously, it was documented that *S. cerevisiae* FB at 40-43 °C supported ethanol yield of 0.40 g/g from 170 g sugars/liter and *S. cerevisiae* F29 yielded only 0.28 g/g product yield under same fermentation conditions as described by Banat et al. 1998. He reported three strains of *S. cerevisiae* Fill, *S. cerevisiae* WR12 and *S. cerevisiae* SIIC which produced 0.45, 0.47 and 0.45 g/ethanol/g sugar at 40-43 °C following growth on 175, 171 and 159 g TS/liter respectively. Abdel-fattah et al. (2000) performed extensive studies with *S. cerevisiae* Fill at 30-45 °C using 160 g TS/liter and got 0.48 g ethanol/g TS and was remarkably good performance of the culture at industrial scale. Their studies on *S. cerevisiae* SIIC gave very low ethanol yield (0.15 g/g) at industrial scale. However *Kluyveromyces marxianus* under similar conditions supported 0.41 g ethanol/g TS at 40-45 °C. Previously Rajoka et al. (2005) isolated a thermostolerant mutant of *S. cerevisiae*, which supported an ethanol yield of 0.49 g/g at 37 -43 °C in 23 liter fermenter. Thus ethanol yield of 0.49g/g by mutant *S. cerevisiae* M-9 from 150-200 g TS/liter at 40-47 °C is quite appreciable. Ethanol production regulatory process was comparable to that exhibited by a *Bacillus* sp Gupta et al., (2004). The production formation a main mot Enhancement in ethanol and Ffase production by the mutant was a consequence of alteration in genes related to all activities, namely hexokinase, or DOG-6Phosphatase as reported in DG' mutants Rincon et al., (2001).

Conclusion:

The agitational intensity of 300 rpm in this bioreactor of 150 liter working volume was found optimal and hyper-production of ethyl alcohol. This productivity is more than 2 fold improved as compare to the wild strain. This thermostolerant yeast strain *Saccharomyces cerevisiae* was utilized at elevated temperatures for testing its thermo-tolerance and productivity of ethyl alcohol. At 37 °C, maximum cell mass produced with concomitant maximum ethanol production (75 g/l). Maximum substrate was consumed in response to the optimized inoculum size (10 % v/v).At the ranges of the temperatures i.e.42-47 °C, the ethanol production decreased in the native organism and a minor decrease was recorded in the sugar consumption too (145 to 140 g/l). However 0.5 to 1.0 % sugar remained in the fermentation broths in these cases. The results of the fermentation at 45 °C and 47 °C by the mutant strain are almost the same with respect to sugar consumption; however the ethyl ethanol production values have been declined as compared to those at 43 °C in 28 h fermentation time. These optimized results of all relevant parameters were followed when the agitational intensity was under observation and under control through a digitally controlled microprocessor. That leads to a conclusion, useful for all sorts of fermentation levels from shake flasks to the commercial scale fermentation in the tropical areas in world experiencing high temperature in the ethanol distilleries that the agitational intensity be under control and monitored through electronically controlled devices.

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