Effects of agitational intensity on production of ethanol using thermotolerant yeast *Sachharomyces cerevisiae* in a digitally controlled pilot scale bioreactor of 150 liters.

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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[1].**
- **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[2,3].**
- **Greater agitation produces more dispersion hence the greater mass transfer rate [4].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 [5].**

- ***Centers***

  Mehran University of Engineering and Technology Jamshoro

  Pakistan Atomic Energy Commission (PAEC), Islamabad.

  Yeast strain of S. cerevisiae

- **Yeast strain of *S. cerevisiae* SAF 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)[6]. 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 hyper-production of ethanol power and used for enhanced ethanol production.**

Materials and Methods

- ***Fermentation***

Fermentation tanks were made up of stainless steel, having working volume of 150 liters.

batch fermentation

43-45 ° C.

Molasses of an optimized brix / concentrations were used.

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

98 100 % total sugars (TS) were consumed

Materials and Methods

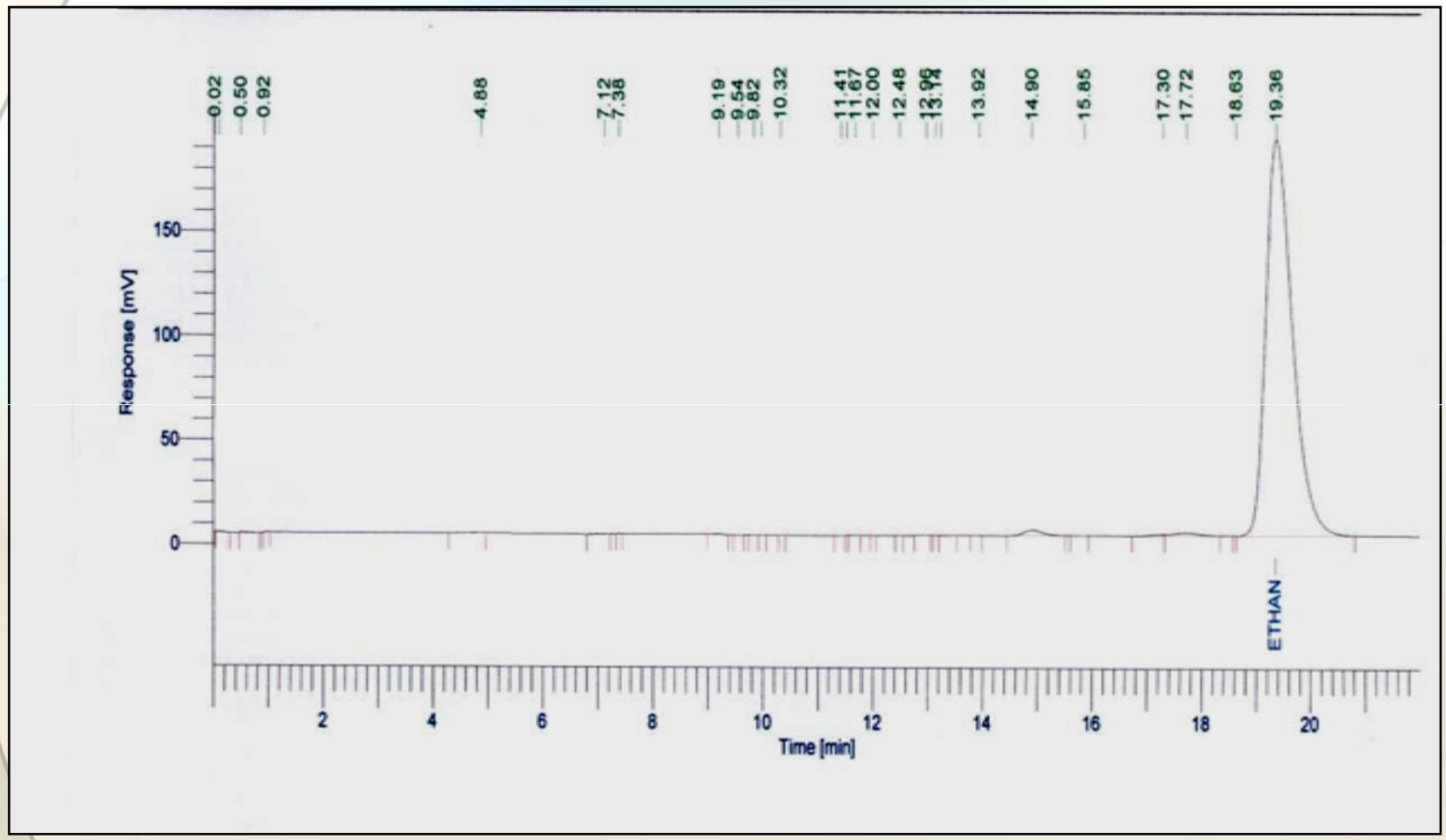

Fig.1: HPLC Chromatogram of fermented molasses containing 15 % TS

- ***Kinetic parameters***

Kinetic parameters were calculated as mentioned in previous study [7].Empirical approach of the Arrhenius was used to describe the relationship of temperature and product formation [8].Following formulae were used for the parameters:

- **Yp/x [Product yield coefficient with respect to cell mass] = dP/dx**
- **Yp/s [Product yield coefficient with respect to substrate] = dP/ds**
- **qp [Product yield coefficient with respect to substrate] = μ x Yp/x**
- **qs [Substrate utilization = μ x /Yx/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.

Results and Discussion

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).

Results and Discussion

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)[9].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[10].

Results and Discussion

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 mmoll-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 [11].

Results and Discussion

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.

Agitational intensity	μ	Q_x	Q_s	Q_p	$Y_{X/S}$	$Y_{P/X}$	$Y_{P/S}$	q_p	q_s
200 rpm	0.22	0.27	6.04	1.7	0.04	6.21	0.28	1.37	5.5
300 rpm	0.29	0.40	6.12	2.54	0.06	7.71	0.50	2.2	4.8
400 rpm	0.24	0.34	6.00	2.79	0.057	8.3	0.46	1.2	4.2
450 rpm	0.20	0.30	4.7	2.16	0.05	6.58	0.35	1.22	4
500 rpm	0.17	0.24	4.1	1.22	0.037	5.97	0.31	1.08	3.6

Results and Discussion

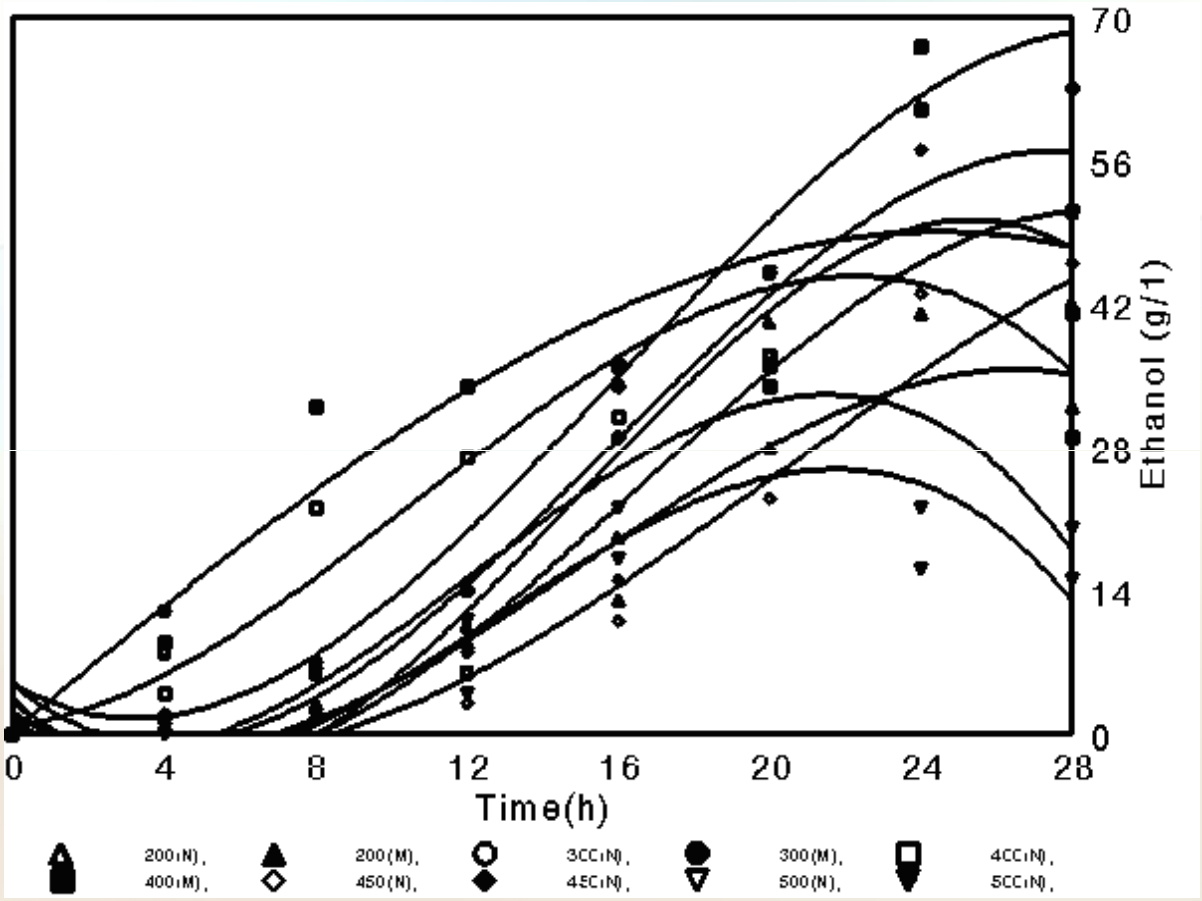

Fig.1: Effect of agitational intensity on ethyl alcohol from Wild and Mutated organisms of *Sachharomyces cerevisiae*. The data given in the figure is an average of the two readings.

Results and Discussion

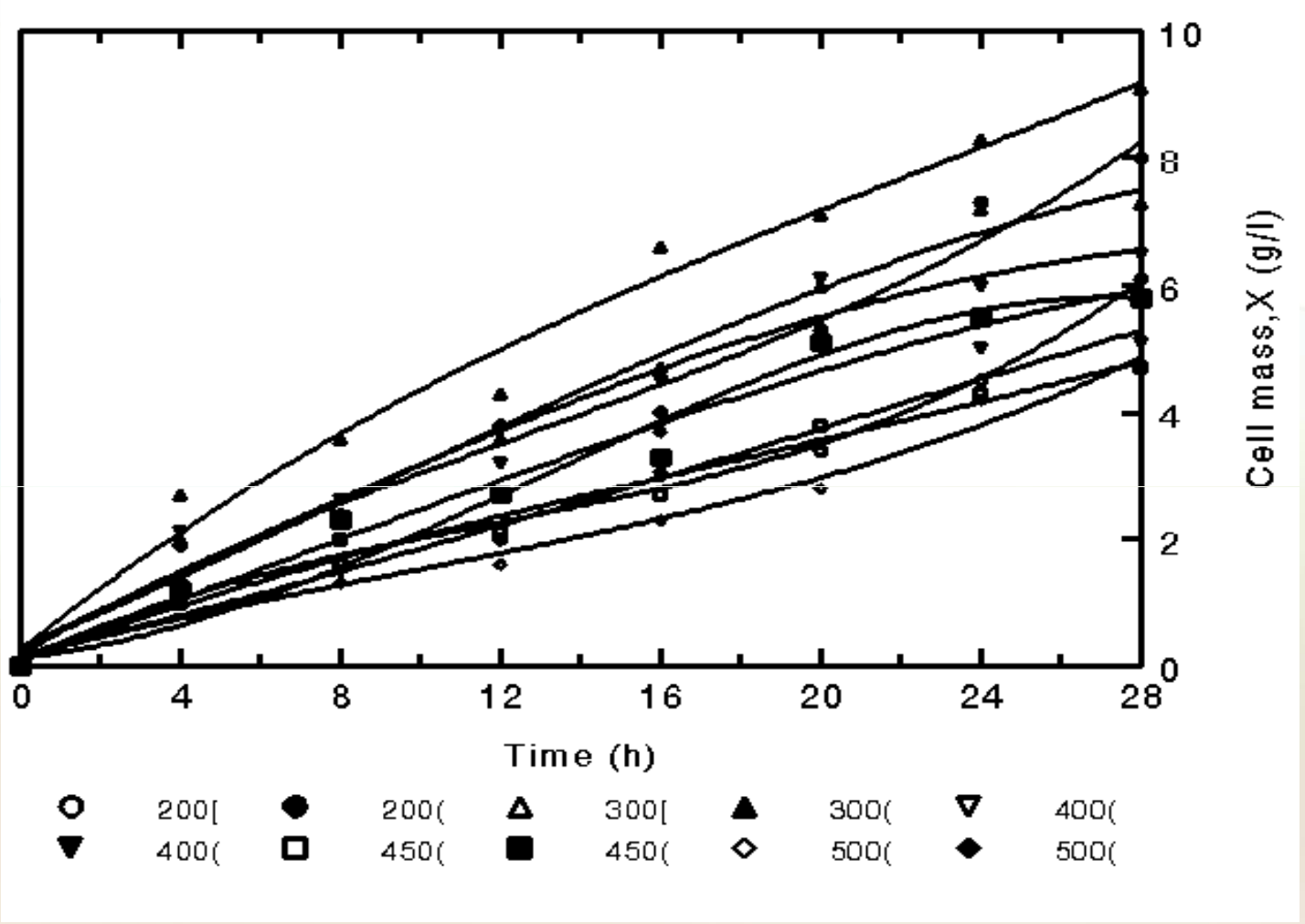

Fig.2: **Effect of agitational intensity on Cell growth of Wild and Mutated strains of *Sachharomyces cerevisiae*. The data given in the figure is an average of the two readings.**

Results and Discussion

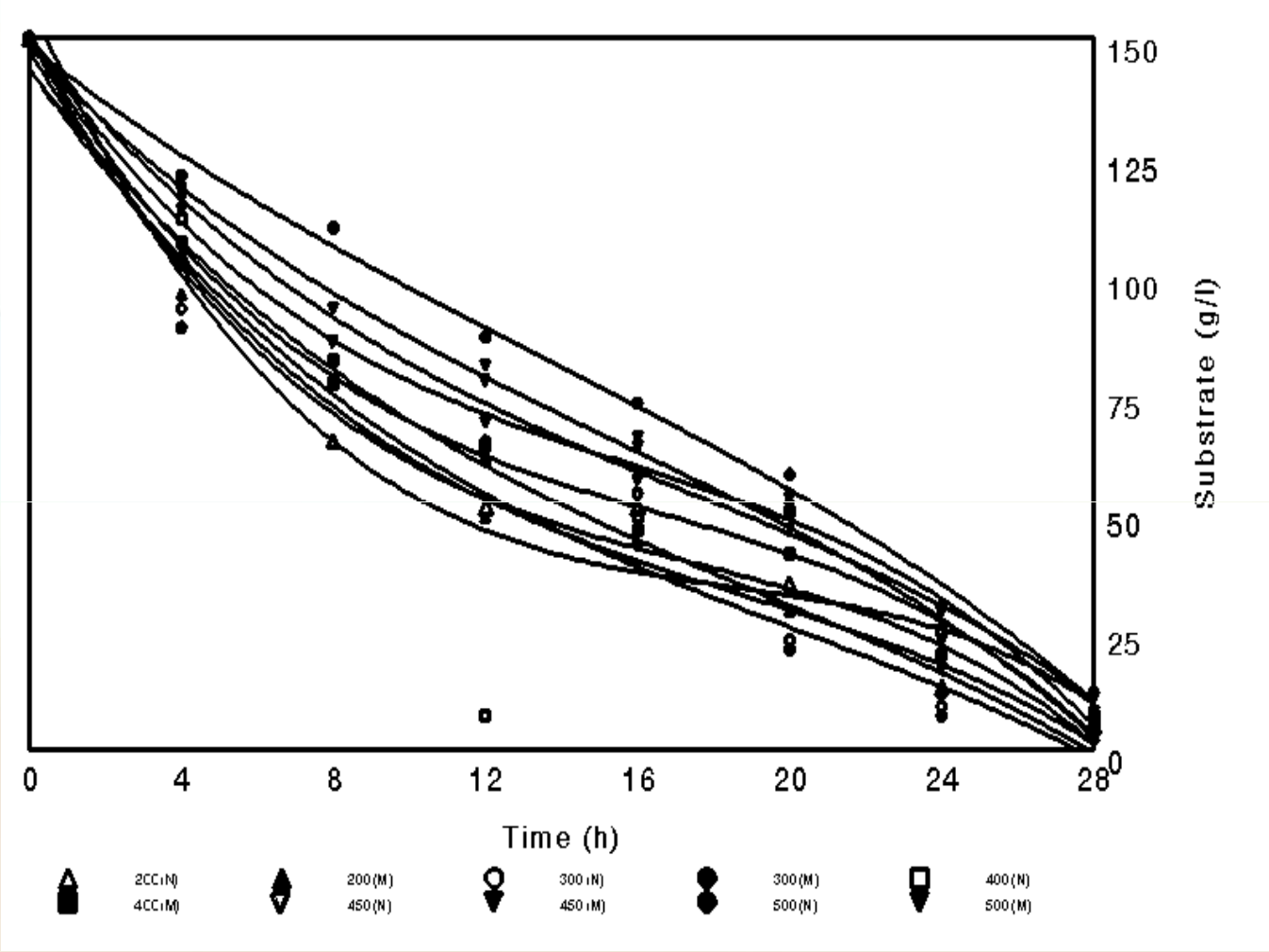

Fig.3: **Effect of agitational intensity on substrate consumption from wild and mutated strains of *Sachharomyces cerevisiae*. The data given in the figure is an average of the two readings.**

Results and Discussion

Qp 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 2)

Results and Discussion

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

Parameters	23 liter	150 liter	S.flask	*F*-value	*p*- value at $p \leq 0.05$
Substrate consumption parameters					
$\mu_m (h^{-1})$	0.34b	0.35a	0.23c	441	0.000
Q_S (g/l h)	4.5b	6.25a	3.8c	204.8	0.0001
$Y_{X/S}$ (g/g)	0.06b	0.066a	0.045c	263.3	0.0000
q_S (g/g h)	5.7a	5.5a	5.6ª	0.12 0	0 .459
Product formation parameters					
Q_P^* (g/l h)	3.38b	4.5a	2.4 c	331.24	0.0001
$Y_{P/S}$ (g/g)	0.49b	0.50a	0.45c	63.0	0.000
$Y_{P/X}$ (g/g)	7.9b	8.30a	8.10c	183.0	0.0001
q_P^* (g/g h)	2.7b	3.0a	1.7c	139.0	0.0002

Results and Discussion

- **Furthermore, the substrate uptake rate was improved 1.39 and 1.64-fold.**

- **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 [12-16].**

- **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.**

Results and Discussion

- **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 [18].**
- **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 DGr mutants [19].**

Conclusions

- **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 thermotolerant yeast strain *Sachharomyces cerevisiae* was utilized at elevated temperatures for testing its thermo-tolerance and ethanol productivity**

- **At the ranges of the temperatures i.e.42-47 ^{o}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).**
- **0.5 to 1.0 % sugar remained in the fermentation broths in these cases.**

Conclusions

- The results of the fermentation at 45 ^{o}C and 47 ^{o}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 ^{o}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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