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Yeast Feed (II) For Enhanced Growth For Ethanol Production

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Abstract: Ethanol is one of the organic solvents produced from various sugar substrates through the action of microbial enzymes. Besides bacteria and yeast e.g. *Saccharomyces cerevisiae* are the most domesticated fungal group for ethanol production. These cells have their characteristic features in terms of shape, size and mode of nutrition. They are able to provide the best possible combination of characteristics for the biological reactions with the given substrates. The intake of feed system-II and water are the essential elements of *Saccharomyces cerevisiae* for its growth and for the industrial processes. Our work highlights the stimulation of the growth of baker's yeast strains, isolated from bakery (Strain S5) and also from the soil samples of the industrial area (Strain S6) respectively. The pure culture was grown on various broth media but the peak of growth enhancement was reported in malt extract, yeast nitrogen base, glucose medium, their characteristics were determined by different parameters and revealed 72 h incubation, 0.5-1 ml inoculum size, 4.0 pH, 30°C, 110 rpm agitation were required for the enhancement of the growth at absorption 600 nm. Tolerance was reported on sun light and moist heat at 30°C but they showed sensitivity to the exposure of UV rays after 15 minutes incubation and the mutagenic chemicals after 72 h. Ethanol and methanol tolerance was reported higher when grown in growth media (Gm 5 and 6) containing optimized concentration of glucose, vitamins, amino acids along with the previously reported feed-I.

Keywords: *Saccharomyces cerevisiae*, feed system, growth features, biofuel synthesis

1. INTRODUCTION

Global change in eighteenth and nineteenth century brought forward the new revolutions in the public and private sector. The recent explosion on the production and subsequent use of biofuels has huge implications for science and future policies. The diversity of biological technologies is approachable with targets including faster growing crops, advanced clarification and pretreatments, suitable enzyme synthesis as well as more modified and improved microorganisms for biofuel synthesis. The need of such advancement helps in the competition for alternative fuels, controlling the oil prices, mounting the climatic changes and supports the carbon-neutral fuels for large scale, low cost production of organic fuels and chemicals to be used as biomass, which is the only domestic and renewable primary energy source for bioproduct industry (Hahn-Hagerdal *et al.*, 2008; Perlack *et al.*, 2005). Nowadays, the petroleum products are thought to be invaluable and are extremely diminishing due to the un-predicted supply that cause the high inflation rate of the petrol demand. The increased rate of petroleum products are due to dearth of resources and this shortage aimed the production of ethanol since very long time from various sources by different workers (Olsson and Hahn-Hegerdal, 1996; Beatriz Palmarola *et al.*, 2005; Mortin *et al.*, 2006). Besides bacteria, the yeasts are truly captivating having more than seven thousands species as reported by Boekhout and Kurtzman (1996), Hawksworth and Mouchacca (1994). The rapid convertion of sucrose-glucose-ethanol with high fermentation property, ethanol tolerance made the best use of *Saccharomyces cerevisiae* in various fermentation processes including the production of wine from apple. Besides, the baker's yeast possesses other properties like nitrogen 6.5-9.3, proteins 40.6-58, carbohydrates 35-45 and lipids 5.0-7.5 percent (Wang *et al.*, 2004; Periyasamy *et al.*, 2009).

2- METHODOLOGY

Saccharomyces cerevisiae cultures were obtained from bakery dough and the soil strain was isolated and investigated according to (Noor and Dahot, 2008; Zakpaa *et al.*, 2009). All test strains were separately inoculated on the surface of Sabraud's dextrose agar, potato dextrose agar, Malt extract, yeast extract, yeast nitrogen base (YNB), glucose medium

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and Czapekdox agar. Samples of both strains were withdrawn at intervals during incubation periods for cell growth, plates were incubated at 37°C for 24 hours and the sub-culturing was undertaken 3 days in fresh media plates (Ghumro *et al.,* 2001; Camacho-Ruiz, *et al.,* 2003).

Growth studies were undertaken following the method of Noor and Dahot (2007). Five replicates of each strain were tested after every 4 hr till 96 hr at different temperatures such as room temperature 37°C in standing incubator and orbital shaking incubator at 110 rpm at A_{600}. Previously fed (I) strains such as S5, S6 were provided various vitamins, sugars and amino acids to bring out the best feed system (II). Total viable count was determined according to the Birch *et al.*, (2003), Noor and Dahot, (2008).

Test strains were separately inoculated in four sets of media containing flasks, 100 ml (v/v), incubated at 30ºC for 10, 20-60 minutes in shaker incubator under the treatment of ultraviolet radiations (260 nm), two sets of 72 hours old culture plates (S5 and S6) were kept under sun light for same duration, two sets of media flasks were inoculated with test strains kept in water bath at various temperatures such as 20-50°C for 15-60 minutes and the fresh inocula of test strains (S5 and S6) were examined in respective media (feed-II) containing ferric chloride, mercuric chloride and phenol at the concentration of 0.2-1.0 ml (v/v), ethanol and methanol at the concentration of 1-15 ml (v/v) and observed at 110 rpm is shaking incubator at 30ºC after 72 hr incubation at A_{600} respectively.

3. RESULTS

Growth studies were undertaken 0-72 h **(Fig.1)** and revealed the enhanced growth of strain S5 as compared to strain S6 after 72 h incubation. **(Fig-5)** in respective growth media Gm 5 and Gm 6 at 0.5 and 1 ml inocula size, pH 4, temperature 30°C at 110 rpm, absorption at A_{600}. During optimization studies of the yeast growth it was observed that the feed (II) viz. sugar (glucose) 1g, vitamins and amino acids 0.1-0.3 mg / 100 ml w/v are required along with feed (I) for the enhanced growth **(Fig.2-4)**. The synthetic growth media (Gm 5 and 6) under optimized concentrations were prepared for enhanced growth of test strains respectively **(Table-1)**. Test strains were examined for tolerance of U.V. radiations **(Fig. 6),** moist heat and various mutagenic chemicals at various concentrations and revealed that, both strain survived for 15 minutes under radiation stress. Strain S5 and S6 showed survival for 20 and 10 minutes on moist heat stress. Studies on mutagenic chemicals revealed the stress tolerance of strain S5 and S6 at 0.2 ml/100 ml concentration of mercuric chloride, ferric chloride and phenol respectively **(Table 2-3)**. Stress tolerance to ethanol was observed at 12 and 10 ml and methanol at 08 and 08 ml v/v of the growth medium Gm5 and Gm6 respectively **(Fig-7-8)**.

Table 1: Chemical composition of synthetic growth medium (GM) feed-I from (Noor and Dahot, 2008) and feed-II* for the maximum growth of S. cerevisiae strain S5 and S6.

Ingredients	Concentration mg / 100 ml	
	Strain S5	Strain S6
Meat extract	3	3
Yeast extract	1	1
Peptone	5	5
Glucose*	100 (1 g)	100 (1 g)
K2HPo4	0.2	0.2
(NH4)So4	0.2	0.4
MnCl2	0.4	****
MgCl2	****	0.6
KOH	0.2	0.4
Nicotinamide*	0.1	****
Riboflavine*	*****	0.2
Thiamine*	0.1	0.2
Glutamic acid*	0.1	0.1
Aspargine*	0.1	0.2
Arginine*	0.2	0.2
Distilled water	100 ml	100 ml
pH	4	4

Table-2: Effect of U V radiations and heat *(water bath temperature* at 20 - 50°C) on the growth of *S. cerevisiae* strain *(S5 and S6)* in growth media (Gm 5 and 6) at pH 4 and 0.5 - 1 ml inoculum size, 30°C 110 rpm after 72 hours incubation (Mean values of three replicates).

Time (minutes)	Effect of *U.V. radiations*		Effect of various temperatures °C (in water bath)							
			20		30		40		50	
	S5	S6	S5	S6	S5	S6	S5	S6	S5	S6
15	1.631	1.329	1.626	1.305	1.634	1.297	1.604	1.313	1.587	1.003
30	1.552	0.286	1.621	1.284	1.638	1.258	1.587	1.284	1.493	0.629
45	1.510	0.231	1.614	1.256	1.644	1.221	1.523	1.229	1.401	0.538
60	1.462	0.170	1.581	1.212	1.653	1.153	1.534	1.183	1.368	0.453

Table 3: Effect of mercuric chloride, ferric chloride and phenol at various concentrations (0.2, 0.4 – 1.0 ml v/v) on the growth of *S. cerevisiae* strain *(S5 and S6 respectively)* in growth media (Gm 5 and 6) at pH 4 and 0.5 - 1 ml inoculum size, 30°C 110 rpm after 72 h incubation (Mean value of three replicates).

Concentration (% v/v)	*Mercuric chloride*		*Ferric chloride*		*Phenol*	
	S5	S6	S5	S6	S5	S6
0.2	1.625	1.089	1.613	1.053	1.618	1.038
0.4	1.616	1.065	1.549	1.027	1.553	1.004
0.6	1.002	1.048	1.485	0.085	1.511	0.073
0.8	0.744	1.029	1.414	0.047	1.469	0.038
1.0	0.550	1.071	1.351	0.023	1.407	0.011

Fig. 1: Determination of initial growth (mean) of S.cerevisiae strain S5 and S6 (inoculum size o.5 and 1.0 ml) at various incubation periods, 30 C, 110 rpm respectively.

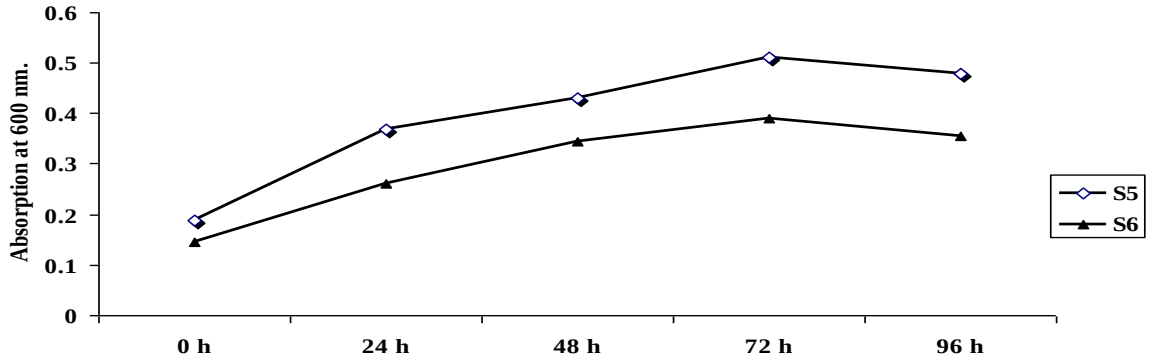


Fig. 2: Determination of maximum growth (mean) of *S.cerevisiae* strain S5 and S6 (inoculum size 0.5 and 1.0 ml) at different sugars at various concentrations (0.4-1.6% w/v)various sugars, 30 C, 110 rpm, 600 nm after 72 hours incubation respectively.

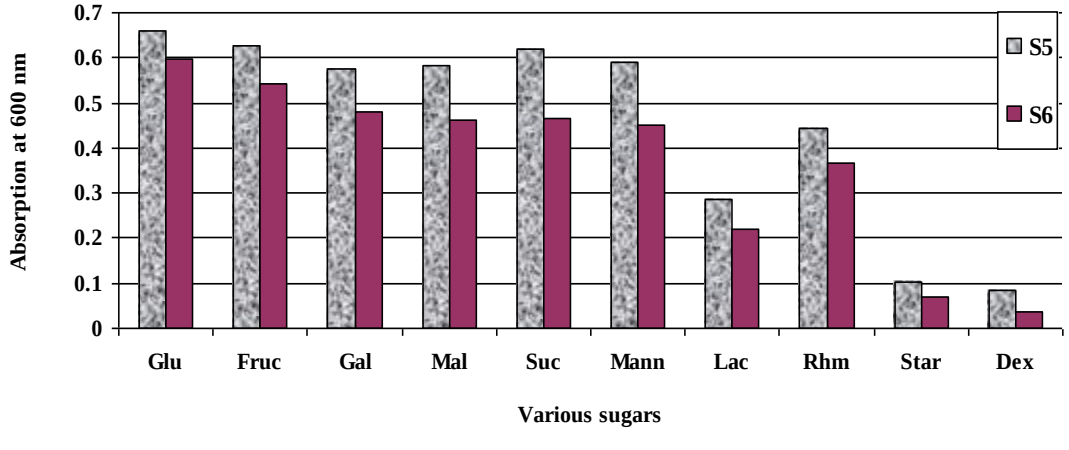


Fig.3: Determination of maximum growth of *S. cerevisiae* strain S5 and S6 (inoculum size 0.5 and 1.0 ml) at various concentrations (0.1 to 0.2% w/v) of different vitamins, 30 C, 110 rpm, absorption 600 nm after 72 h incubation respectively.

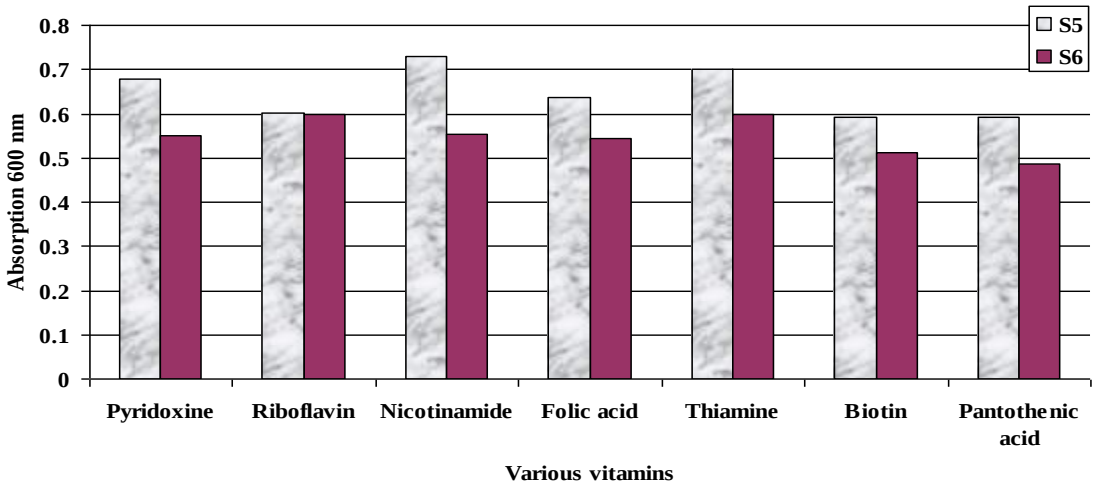


Fig. 4: Determination of maximum growth (mean) of *S.cerevisiae* (inoculum size 0.5-1.0 ml) at various concentrations (0.1-0.3% w/v) of different amino acids at 30 C, 110 rpm, absorption 600 nm after 72 h incubation respectively.

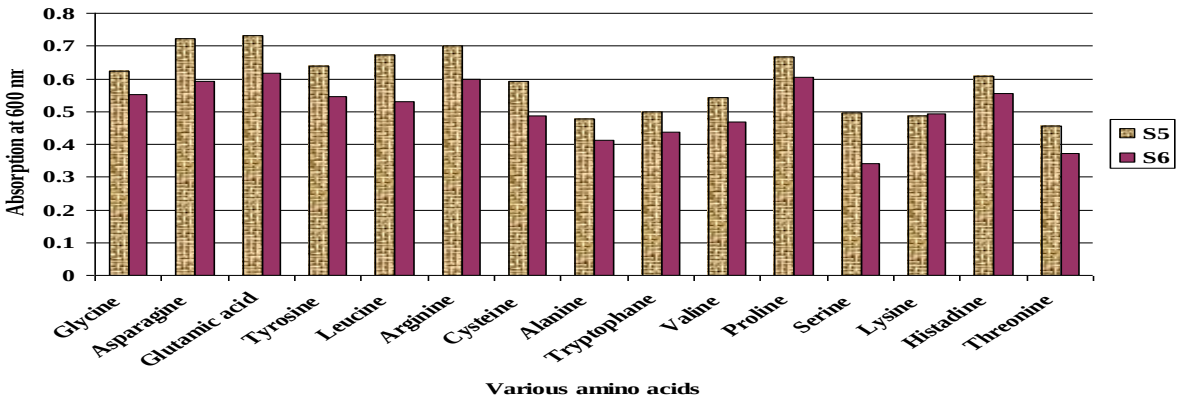


Fig.5 Camparasion of final enhanced growth of S.cerevisiae strain S5 and S6 on Gm5 and Gm6 after 72 h at pH 4, 30 C, 110 rpm, 600 nm.

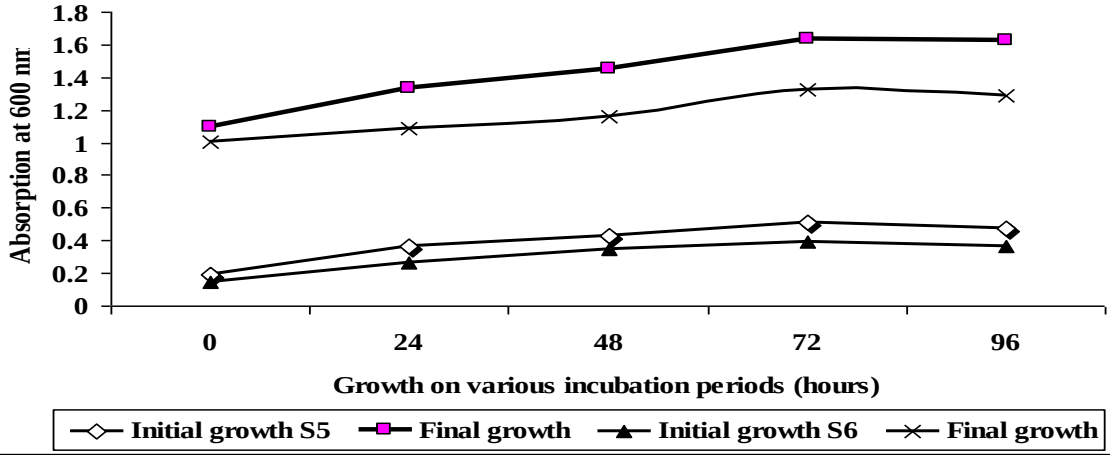


Fig.6: Determination of the effect of Ultravoilet radiations of the final growth S. cerevisiae strain S5 and S6 at various time durations in Gm % and Gm6 respectively

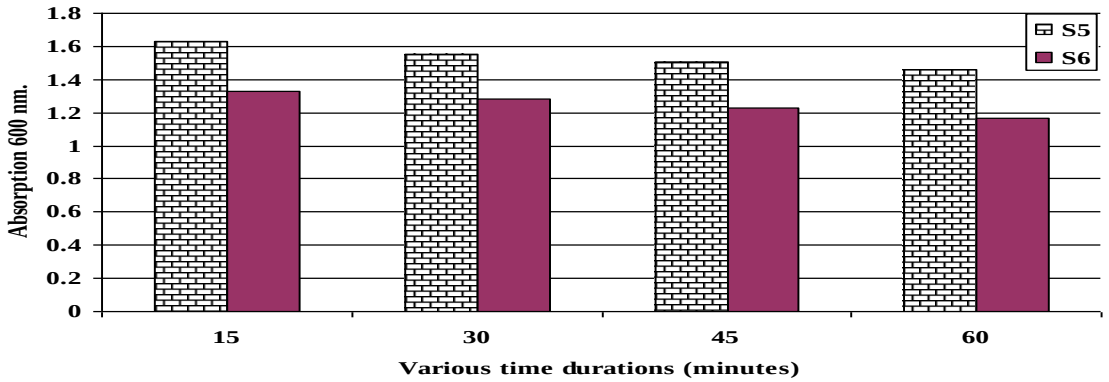


Fig. 7: Determination of the effect of various concentrations of ethanol on the growth of S.cerevisiae strain S5 and S6 in Gm5 and Gm6 after 72 h, 110 rpm, pH 4, 600nm respectively

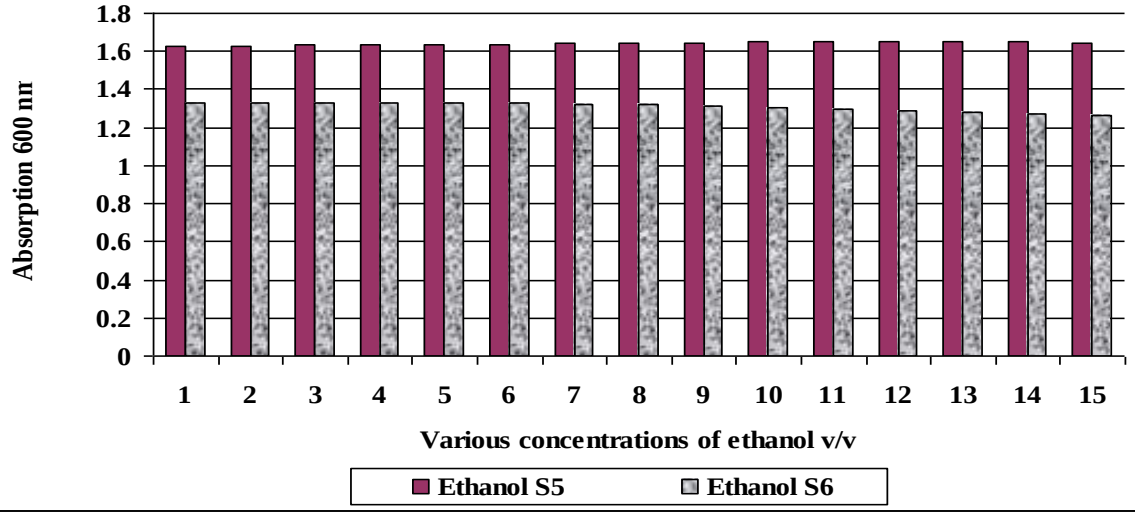
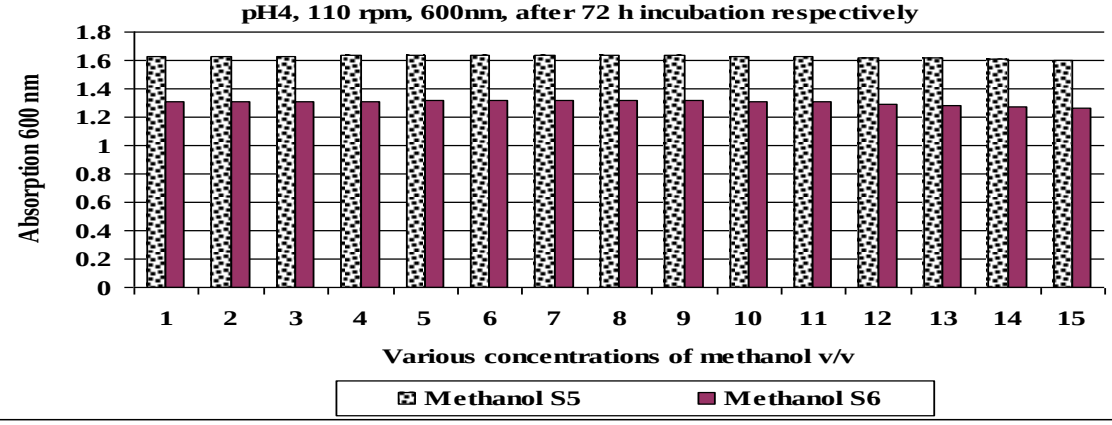


Fig. 8: Determination of the effect of various concentrations ml/100ml of methanol on the growth of S.cerevisiae strain S5 and S6 in Gm5 and Gm6 at pH4, 110 rpm, 600nm, after 72 h incubation respectively



4. DISCUSSIONS

Ethanol fermentation by yeast from renewable resources is an integral part of the current sources of energy (Moritz and Sheldon, 1996). The main emphasis of increased growth of test strains is to find out the best strain and development of new strain for ethanol fermentation. Yeast cultures in dehydrated, in-active form causes damage cell membrane and modify the chemical nature of various constituents of the cell. Re-hydration and activation process supports the moisture level for the growth, faster metabolic activity, higher cellular mass, rapid sugar utilization specially glucose due to the action of cytoplasm glucosidase (Alpas *et al.,* 1996; Edgerton, 2001; Noor *et al.,* 2003: Wang *et al.,* 2004). The seed medium of yeast growth i.e. malt extract yeast extract peptone glucose agar was modified where peptone was replaced with Yeast Nitrogen Base to prepare Malt extract Yeast extract Yeast Nitrogen Base Agar supports growth due to the presence of yeast nitrogen base as a sole nitrogen source as well as other growth factors for carbon assimilation and fermentation Walker, (1997).

Starter's growth in log phase with rapid metabolic activity of strain S5 and S6 were observed at 72 h. at specific volume of inocula, which competes for the intake of food material by the supplementation of the other growth factors (feed-II). Our results showed that the supplementation of growth enhancing factors increased the growth rate and tolerance of both test strains to the stressed environment as reported by Noor and Dahot, (2008). Variation in growth may be due to weak acids that could be transported in un-dissociated form and act by dissipating plasma membrane proton gradients plus medium chain fatty acids cause cellular break down that's why the growth increases due to increase in pH (Stanford and Anslow, 1996; De Vasconcelos *et al.,* 1998; Nigam, 1999; Periyasamy *et al.,* 2009). Oxygen and temperatures are the main factors that support the inoculum development (Noor *et al.,* 2003). Higher temperatures affects the growth due to the inability of yeast's regulatory mechanism for the tolerance. These observations are in accordance with Coote *et al.,* 1991; Diwany *et al.,* 1992; Shah and Hameed, 2004; Noor and Dahot, 2008). Growth enhancement is also due to the uniform rotations of the orbital shaker, which provides oxygen needed for mitochondrial cyclic metabolism the equal concentrations of the nutritional ingredients to all cellular components Walker, (1997).

Toxicity is reported when the nutritional requirements reached beyond the optima (Walker and Maynard, 1997). Significance of the inorganic substances (feed-I) are already discussed in Noor and Dahot (2008). Sugars provide energy and carbon is less concentration may support small proportion of uptake into yeast cells (Walsh *et al.,* 1994).Genes provides hereditary traits and are responsible for increased growth as reported by Andre, (1995), Ozcan and Johnston, (1995) encoding the transportation mechanism by the stimulation of mitochondrial enzymes (Noor and Dahot, 2008). The utilization of disaccharide sugars is reported through active proton-symport mechanisms either are intact or firstly hydrolysed out with the yeast plasma membrane prior to transport of the component monosaccharide whereas glucose and galactose transporters in *Saccharomyces cerevisiae* act by facilitated diffusion and the growth on polysaccharides such as starch is un known due to the lack of amylase production ability (Sreekrishna and Dickson, 1985; De Juan and Lagunas, 1986; Walker, 1997).

Amino acids play a specific structural role, the mixtures of amino acids are sequentially assimilated and immediately transported whereas some are transported gradually and few are transported after an appreciable lag time. Vitamins have catalytic and structural role, which helps in acetylation, decarboxylation, redox reactions, carboxylase-catalyzed reactions and transfer of methyl groups (Walker, (1997).

During growth the stressed environment show inhibitory effects when they are exposed beyond the optima. Heat stable shock treatment of test strains induces the synthesis of particular heat-shock proteins (Ruis and Schuller, 1995; Walker, 1997). Reduced yeast growth may be due to the activation of transcriptional control elements. Ultraviolet radiations causes damage to the yeast's DNA including the dimerization, nicks and lesions (Hino *et al.,* 1990; De Virgillo *et al.,* 1994; Walker, 1997). Chemical stresses may cause cellular damages to acetaldehyde. Ethanol in greater concentration causes the increased membrane fluidity and decreased membrane integrity in yeasts and destruction of cellular enzymes and kills the yeast cells due to the accumulation of dissolved toxic products (Watson and Cavicchioli, 1983). Methanol suppresses the yeast growth at very low concentration because the yeasts are unable to metabolize by an oxygen-dependent oxidase to formaldehyde, which is then converted into dihydroxyacetone by DAH synthase enzyme (Noor and Dahot, 2008).

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