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Enhanced Growth Biotechnology of Yeast for Alcoholic Fermentation

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Abstract: Fermentation is the new mode of achieving new and finished products from the microbial sources within the required parameters. It is widely used to make variety of useful products by the action of microbial enzymes. This study deals with the growth parameters of *S. cerevisiae* strains for its maximum/enhanced growth which is required for the higher yield of the desired product e.g. alcohol. The results revealed that, under the optimized parameters such as temperature, agitation speed the growth mass of the test strains increases at 120 rpm, 30°C, pH 4.5, A_{600} after 72 h post incubation in Yeast Extract, Peptone, Glucose (YEPG) broth supplemented with Yeast Nitrogen Base (YNB).

Key words: Growth, optimization parameters, *S. cerevisiae*, fermentation

INTRODUCTION

The importance of the industrial production of fine chemicals and pharmaceuticals by fermentation has increased with the introduction of recombinant microorganisms. *Saccharomyces cerevisiae* is frequently the yeast of choice that are rich in glycogen, enzymes, easily harvested, possessing low nucleic acid content and high lysine activity and they are also able to grow in acidic pH. They have the special virtue of possessing particularly efficient aerobic (respiro-fermentation) and anaerobic capabilities for fermentation processes[1-4] and are unicellular eukaryote, possesses basic function, common to wide variety of eukaryotes. Genetic operations on the cell are well established and in recent years much work has been done by using yeast as a model system. They are not as ubiquitous as the bacteria in the natural environment, but nevertheless yeast can be isolated from terrestrial, aquatic and aerial samples and they are also associated with plants, animals and insects. The fermentative activities have been exploited by human for millennia in the production of beer, wine and bread that's why, yeast are called the most important domesticated microorganisms[4-6].

This presentation has undertaken to screen out the highest growth producing strain of test microorganisms for the future production of the high yield of alcohol which is commonly used in laboratories and also promote the production of ethanol of fuel grade in order to overcome the high prices of fuel in our country.

MATERIALS AND METHODS

The entire work was undertaken in the Microbiology Research laboratory at Quaid-e-Azam University, Islamabad, Pakistan, During this presentation, previously activated (growth i.e. A_{600}, total viable count i.e. cells mL^{-1}, growth rate-K, total cell dry weight) Saccharomyces cerevisiae strains KA-1 ($25x10^4$, 0.0279, 0.101), AAN-2 ($27x10^4$, 0.0304, 0.067), ASN-3 ($27x10^4$, 0.0330, 0.074) and HA-4 ($28x10^4$, 0.0331, 0.090)[7-10] and were re-activated by the method[10] and Optical Density (OD) was obtained at A_{600}.

Finally, 1 mL of each activated broth cultures were inoculated in 100 mL of YEPG with 0.5, 0.3, 01% concentration, respectively in 400 mL Erlenmeyer flasks. Initial OD was determined later the medium was supplemented with 01% concentration of YNB. Flasks were allowed at various agitation speeds (100-150 rpm.) of rotary shaking incubator, temperatures (28-36°C) and examined for the growth (OD) at A_{600} after every 6 h and for the growth rate (K) and total cell dry weight after every 24 h.

RESULTS AND DISCUSSION

Activated cultures of *S. cerevisiae* strains were re-activated individually and later the mixed culture of *S. cerevisiae* strains KA-1, AAN-2, ASN-3 and HA-4 was prepared in Wickerham's broth, allowed for 72 h at 30°C, 120 rpm, 4.5 pH, A_{600} and revealed OD 0.23. Final

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Table 1: Determination of maximum growth (A_{600}) of mixed culture of *Saccharomyces cerevisiae* strains at various temperatures, growth rate, total dry cell weight, at 100 rpm, pH 4.5 after 96 h post incubation in YEPG broth supplemented with YNB

Incubation	Various temperature (°C)					Growth	Total cell
period (h)	28	30	32	34	36	rate (K)	dry weight
6	0.14	0.19	0.18	0.14	0.12		
12	0.20	0.28	0.26	0.21	0.18		
18	0.26	0.37	0.34	0.28	0.24		
24	0.32	0.46	0.42	0.35	0.30	0.0170	0.022
30	0.38	0.55	0.50	0.42	0.36		
36	0.44	0.64	0.58	0.49	0.42		
42	0.50	0.75	0.66	0.56	0.48		
48	0.55	0.86	0.75	0.63	0.54	0.0237	0.043
54	0.67	0.97	0.84	0.71	0.60		
60	0.69	1.08	0.93	0.79	0.66		
66	0.76	1.19	1.02	0.87	0.73		
72	0.83	1.30	1.11	0.95	0.80	0.0286	0.069
78	0.78	1.25	1.06	0.88	0.69		
84	0.70	1.17	1.00	0.80	0.57		
90	0.62	1.09	0.87	0.71	0.45		
96	0.55	1.00	0.75	0.62	0.33	0.0254	0.049

Table 2: Determination of maximum growth (A_{600}) of mixed culture of *Saccharomyces cerevisiae* strains at various temperatures, growth rate, total dry cell weight, at 110 rpm, pH 4.5 after 96 h post incubation in YEPG broth supplemented with YNB

Incubation	Various temperature (°C)					Growth	Total cell
period (h)	28	30	32	34	36	rate (K)	dry weight
6	0.15	0.20	0.19	0.17	0.15		
12	0.21	0.30	0.27	0.24	0.21		
18	0.27	0.38	0.35	0.31	0.27		
24	0.33	0.46	0.43	0.38	0.33	0.0165	0.023
30	0.39	0.54	0.51	0.44	0.39		
36	0.47	0.65	0.59	0.52	0.44		
42	0.55	0.76	0.67	0.59	0.51		
48	0.63	0.87	0.78	0.66	0.57	0.0226	0.048
54	0.71	0.98	0.89	0.73	0.63		
60	0.79	1.09	1.00	0.81	0.70		
66	0.87	1.22	1.08	0.89	0.77		
72	0.95	1.35	1.16	0.97	0.84	0.0272	0.071
78	0.91	1.26	1.09	0.90	0.78		
84	0.88	1.19	1.01	0.83	0.67		
90	0.80	1.03	0.90	0.75	0.55		
96	0.72	0.92	0.82	0.66	0.41	0.0243	0.056

Table 3: Determination of maximum growth (A_{600}) of mixed culture of *Saccharomyces cerevisiae* strains at various temperatures, growth rate, total dry cell weight, at 120 rpm, pH 4.5 after 96 h post incubation in YEPG broth supplemented with YNB

Incubation	Various temperature (°C)					Growth	Total cell
period (h)	28	30	32	34	36	rate (K)	dry weight
6	0.16	0.22	0.22	0.19	0.16		
12	0.24	0.35	0.33	0.30	0.22		
18	0.31	0.48	0.45	0.37	0.29		
24	0.40	0.61	0.57	0.44	0.36	0.0184	0.026
30	0.48	0.76	0.69	0.50	0.41		
36	0.56	0.91	0.81	0.56	0.50		
42	0.64	1.06	0.95	0.62	0.58		
48	0.75	1.21	1.09	0.69	0.65	0.0257	0.056
54	0.86	1.37	1.23	0.76	0.73		
60	0.97	1.53	1.37	0.84	0.81		
66	1.08	1.70	1.51	0.93	0.90		
72	1.19	1.87	1.66	1.08	1.00	0.0310	0.083
78	1.14	1.84	1.62	1.06	0.88		
84	1.08	1.79	1.55	1.00	0.80		
90	1.01	1.73	1.50	0.93	0.69		
96	0.94	1..66	1.42	0.81	0.55	0.0289	0.058

Table 4: Determination of maximum growth (A_{600}) of mixed culture of *Saccharomyces cerevisiae* strains at various temperatures, growth rate, total dry cell weight, at 130 rpm, pH 4.5 after 96 h post incubation in YEPG broth supplemented with YNB

Incubation	Various temperature (°C)					Growth	Total cell
period (h)	28	30	32	34	36	rate (K)	dry weight
6	0.14	0.20	0.19	0.16	0.14		
12	0.22	0.31	0.28	0.25	0.19		
18	0.30	0.42	0.39	0.35	0.26		
24	0.38	0.53	0.44	0.42	0.34	0.0185	0.023
30	0.45	0.66	0.52	0.48	0.40		
36	0.54	0.79	0.61	0.55	0.46		
42	0.62	0.89	0.70	0.64	0.50		
48	0.71	1.02	0.77	0.71	0.53	0.0262	0.050
54	0.80	1.16	0.85	0.79	0.58		
60	0.89	1.29	1.00	0.88	0.66		
66	0.98	1.43	1.11	0.98	0.73		
72	1.07	1.57	1.24	1.09	0.80	0.0304	0.076
78	1.00	1.50	1.18	1.04	0.75		
84	0.91	1.44	1.12	1.00	0.69		
90	0.83	1.38	1.10	0.88	0.60		
96	0.71	1.31	1.01	0.76	0.52	0.0281	0.048

Table 5: Determination of maximum growth (A_{600}) of mixed culture of *Saccharomyces cerevisiae* strains at various temperatures, growth rate, total dry cell weight, at 140 rpm, pH 4.5 after 96 h post incubation in YEPG broth supplemented with YNB

Incubation	Various temperature (°C)					Growth	Total cell
period (h)	28	30	32	34	36	rate (K)	dry weight
6	0.12	0.18	0.16	0.13	0.10		
12	0.20	0.27	0.25	0.20	0.14		
18	0.28	0.36	0.34	0.26	0.17		
24	0.36	0.45	0.43	0.31	0.20	0.0174	0.022
30	0.44	0.54	0.52	0.37	0.23		
36	0.52	0.63	0.61	0.43	0.29		
42	0.59	0.72	0.70	0.49	0.34		
48	0.66	0.83	0.78	0.55	0.37	0.0209	0.030
54	0.73	0.94	0.88	0.60	0.41		
60	0.80	1.05	0.98	0.65	0.45		
66	0.85	1.16	1.08	0.71	0.51		
72	0.90	1.27	1.19	0.78	0.59	0.0289	0.068
78	0.86	1.22	1.17	0.74	0.56		
84	0.80	1.13	1.09	0.65	0.50		
90	0.71	1.08	1.00	0.60	0.43		
96	0.62	1.00	0.88	0.51	0.35	0.0261	0.033

Table 6: Determination of maximum growth (A_{600}) of mixed culture of *Saccharomyces cerevisiae* strains at various temperatures, growth rate, total dry cell weight, at 150 rpm, pH 4.5 after 96 h post incubation in YEPG broth supplemented with YNB

Incubation	Various temperature (°C)					Growth	Total cell
period (h)	28	30	32	34	36	rate (K)	dry weight
6	0.09	0.15	0.13	0.09	0.07		
12	0.15	0.22	0.16	0.11	0.08		
18	0.21	0.29	0.18	0.14	0.10		
24	0.27	0.36	0.21	0.17	0.12	0.0170	0.019
30	0.29	0.43	0.26	0.20	0.14		
36	0.33	0.47	0.29	0.24	0.17		
42	0.39	0.57	0.33	0.28	0.20		
48	0.45	0.64	0.37	0.33	0.24	0.0230	0.038
54	0.51	0.71	0.41	0.38	0.28		
60	0.55	0.75	0.46	0.45	0.31		
66	0.59	0.81	0.51	0.49	0.35		
72	0.64	0.86	0.58	0.55	0.40	0.0265	0.061
78	0.63	0.84	0.52	0.52	0.34		
84	0.58	0.78	0.45	0.45	0.28		
90	0.51	0.73	0.40	0.38	0.21		
96	0.46	0.66	0.33	0.30	0.10	0.0223	0.036

inoculation in YEPG medium revealed OD 0.71. After adding YNB in YEPG broth the optical density increased and indicated the highest growth (OD) 1.87 at 600 nm, growth rate (K), 0.083 total cell dry weight, respectively at agitation speed 120 rpm, temperature 30°C, pH 4.5, after 72 h post incubation in rotary shaker incubator (Table 1-6).

From the above results it is highlighted that, the microbial strains as laboratory animals can be activated for their higher growth rate and these are widely used in developing countries in the field of biotechnology to produce the desired products[11]. In the recent era, microorganisms including bacteria, yeast and the moulds are frequently employed to get the known product through their enzymatic activities. Yeast can be cultivated on malt medium obtained from sprouted grains as the carbohydrate e.g. maltose as carbon and energy sources. This is because the industrial applications of yeast such as, brewing, bread-making and the production of distilled beverages or fuel ethanol, rely on their ability to ferment α-glucosides such as maltose.

Addition of peptone from Soya bean meal has significance for strain maintenance as a proteinaceous substance [3,12]. Yeast extract supplemented with peptone and glucose (as in YEPG) is commonly employed for the maintenance and growth of most yeasts. YNB is commercially available chemically defined medium that contains ammonium sulfate and asparagine as nitrogen sources, together with mineral salts, vitamins and trace elements, which are used for the metabolic functions and growth enhancement factors[4].

It is also reported that, among the cationic yeast nutrients potassium, calcium and zinc are involved in structural and the enzymatic regulatory activities during growth and metabolism. Potassium is mainly involved in osmoregulation, charge balancing and in the regulation of divalent cation and phosphate uptake into the yeast cell. Zinc acts as co-factor for the activity of metalloenzymes e.g. alcohol and aldehyde dehydrogenases and the cysteine desulfydrase. It also activate riboflavin synthesis, increase protein content in fermentation yeast and also help in the uptake of maltose into the cell if added into the medium[13].

Hydrogen ion concentration (pH) 4.5 was maintained with HCl because most yeast grow well between pH 4.5 and 6.5. Media acidified with organic acids are more inhibitory to yeast growth than are media acidified with mineral acids like HCl. This is because undissociated organic acids can lower intracellular pH following their translocation across the yeast cell membrane. Intracellular pH is regulated with in relatively narrow ranges in growing yeast cells (around pH 5.00 in *S. cerevisiae*) mainly through the action of the plasma membrane proton-pumping ATPase.

Most species thrive in warm, dilute, sugary, acidic and aerobic environments. Most of the laboratory and industrial yeast grow best at 20-30°C and give higher cellular mass. Temperature is an essential parameter that affects the ups and down of the yeast growth and governs its metabolism. An initial temperature enhanced the growth but as the nutrients were added in propagating medium and incubation was increased from 24-72 h the highest cellular yield was determined at 30°C. It is due to the increased death rate at elevated temperature beyond their optima because the yeast cells growing quickly in a glucose rich medium are more sensitive to heat than others. Temperature shock leads to alteration in physical states of both membrane's composition and intracellular water resulting in increased permeability.

The agitation speed provides the maximum and uniform supply of nutritional materials provided in the medium and also the supply of oxygen which is an essential step to achieve the proper metabolic activity by the activity of mitochondria[4,14,10].

The biotechnological parameters for the maximum growth of previously activated mixed culture of *S. cerevisiae* strains KA-1, AAN-2, ASN-3 and HA-4 were under taken to screen out its efficiency for higher yield of bioethanol from different sugar sources. It is concluded that, mixed culture shows a lower growth (OD), total viable count/cells mL^{-1}, growth rate (K) and total cell dry weight than the strains ASN-3 and HA-4 individually; at 120 rpm, 30°C, pH 4.5 after 72 h incubation when inoculated in YEPG medium supplemented with YNB. It is concluded that, strains ASN-3 and HA-4 are efficient strains and the good candidates for bioethanol fermentation from molasses, dates and other sugar sources.

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