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SCP production from *Saccharomyces cerevisiae* isolated from mangrove sediment

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Saccharomyces cerevisiae,
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Abstract

In the present study a total of 23 morphologically distinct yeast strains were isolated from the sediment samples collected from Pichavaram mangroves. The potent isolate PMS9 was selected for further study and it was identified as *Saccharomyces cerevisiae*. The potential strain was kept for mass scale culture with the optimized growth parameters such as pH-6, 30^{o}C 0% NaCl, 48 hrs incubation, sucrose as carbon source (3%), and 0.5% yeast extract as nitrogen source. The total biomass yield was 5.14g/L in which the crude protein, total carbohydrate and total nucleic acid content of biomass were respectively 40.5%, 30%, and 5.8% before nucleic acid reduction whereas after nucleic acid reduction they were found be 44.5%, 30% and 1% respectively.
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Introduction

Proteins are important dietary constituent and the major structural components of the body. With the increased growth of population and increased demand food supply leads to an increase in the number of hungry and chronically malnourished people. During 1934-1938 the less developed areas of the world, Asia, Africa and South America, were the main exporters of grain to the developed world. Since 1948 the food flow has reversed, from the developed world to the less developed, mainly due to the rate of growth of the world's population which was much higher in the less developed countries (Tannenbaum and Wang, 1975).

This situation has created a demand for an alternative protein rich food sources. SCP production is one of such an alternative technologies in which dead, dry cells of microorganisms such as yeast, bacteria, fungi and algae are being used as either for livestock or for human consumption to overcome the food shortage in the less developed countries in the future. SCP, known as “microbial protein” is easy to obtain in crude form microbes and is highly nutritive. Hence the present study was on isolation of a potential protein rich marine yeast from Pichavaram mangrove sediment sample for the production of SCP.

Materials and Methods

Isolation of yeast strains from mangrove sediment

For isolation of yeast from mangrove sediment, samples were collected from Pichavaram mangrove sediment and were kept in ice-box and transported to the laboratory. The samples were processes as early as possible. Exactly 1g of sediment sample was added to 99 ml blanks and further serially diluted up to 10^{-7}. After serial dilution the samples were transferred into Sabouraud glucose agar plates using spread plate technique and incubated for 3 days at room temperature. Colonies with different morphology were selected pure cultured and stored in SGA slants for further study. Potential strains for SCP production were selected based on their growth (OD at 600nm) and protein content.

Identification of yeast strains

The isolated yeast colonies were identified based on colony color, shape, texture, microscopic morphology, physiological, biochemical tests especially various sugar assimilations (Kurtzman and Fell, 1998 and Barnett *et al.,* 2000).

Optimization for growth

The shake-flask culture of potential strain was optimized for the effect of different environmental parameters like incubation period, pH, temperature, salinity and different carbon and nitrogen sources on the growth of the potential strain. While optimizing the pH for the potential strain, SGB was prepared with different pH in the range pH – 4-9 (4, 5, 6, 7, 8 and 9). Then a loopful of culture was inoculated to each tube and were kept in a shaker (120 rpm) for incubation. The absorbance was measured at 600 nm with an interval of 6 hrs. Similarly, various temperatures like 25^{o}C, 30^{o}C, 35^{o}C, 40^{o}C and 45^{o}C, different salt concentration in the range 0-2.5 % (0%, 0.5%, 1%, 1.5%, 2% and 2.5%). The effect of incubation period on growth of the yeast strains was studied by varying the incubation period from 0 to 60 hrs. Different carbon sources such as glucose, maltose, fructose, sucrose and starch were added in the medium in separate flasks at the concentration of 4% and incubated. Different concentration of ideal carbon source (0.5 – 2.5%) was maintained in the medium and incubated. Different nitrogen sources such as beef extract, yeast extract, peptone, ammonium nitrate, ammonium sulphate and potassium nitrate were added in the medium separately at the concentration of 0.5% in the medium and incubated. Different concentration of yeast extract as nitrogen source (0.1-1.0%) was maintained in the medium and incubated at 35^{o}C. Growth was assessed at 600 nm for every 6 hrs. All optimization studies were done at 120 rpm.

Reduction on nucleic acid in yeast protein

Reduction of nucleic acid and extraction of protein from yeast cells with sodium hydroxide was carried out according to the method described by Herbert *et al.,* 1971. 20 ml of 1 N NaOH was added to 5 g dried yeast cells. Extraction of protein was performed in a boiling water bath for 10 min. This was then followed by cooling in cold water. The solution was centrifuged and the supernatant was used for the estimation of RNA and DNA.

Mass scale culture in shake flask

The potential strain identified as *S.cerevisiae* was kept for mass scale culture with the optimized growth parameters such as pH-6, 30^{o}C, 0% NaCl, 48hrs incubation, 3% sucrose as carbon source and 0.5% yeast extract as nitrogen source. After incubation harvest was done and cells were separated by centrifugation at 10, 000 rpm for 30 min. The weight of biomass was gravimetrically done and expressed on dry weight basis g/L.

Preparation of cell extract

The cell extract was prepared as per the procedure described by Ruiz *et al.,* 1999. Cell pellet was washed twice with normal saline (0.1% NaCl) and suspended in phosphate buffer (0.1 M, pH 7.0) in 1:1 ratio. It was sonicated at 0^{o}C for 2 min at an interval of 30sec with LABSONIC U sonicator (133 V, 0.5 repeating cycles per sec.) Unbroken cells were removed by centrifugation at 4^{o}C for 20 min. at 10,000 rpm. The crude preparations were used for further analysis.

Estimation of protein, carbohydrate and nucleic acids

Protein

The method of Lowry *et al.*, 1951 was adapted for the estimation of total protein. 10 mg of dried yeast cells were thoroughly homogenized with 1 ml of deproteinising agent (10% TCA) by keeping the tubes in an ice bucket and samples were centrifuged for 20 min. at 3000 rpm. The precipitate thus obtained was used for protein estimation. The precipitate was dissolved in 2 ml in NaOH and to 1 ml of this solution, freshly prepared 5 ml of alkaline reagent was added. This was kept at room temperature for 10 min., after which 0.5ml of 1N Folin-ciocalteu reagent was added and mixed rapidly. The standard stock solution was prepared using BSA (Bovine Serum Albumin) at a concentration of 25 mg/5ml 1N NaOH. Different dilutions in the range of 0.25-2.5 mg/ml were prepared from this stock solution, the alkaline reagent and Folin-phenol reagent were added as in the case of yeast dried samples. A blank was prepared with 1 ml 1N NaOH and treated the same way as above.

All the test tubes were kept for 30 min. at room temperature and the optical density (OD) of the blue color developed was measured against the blank at 660 nm.

$$\text{Protein \%} = \frac{\text{Standard value x OD of the sample}}{\text{weight of the sample}} \times 100$$

Carbohydrate

The total carbohydrate was estimated by phenol-sulphuric acid method of Dubois *et al.*, (1956). 5 mg of dried yeast cells were taken for carbohydrates analysis. The dried yeast cells were taken in a test tube and 1 ml of phenol (5%) and 5 ml concentrated H_2SO_4 were added in quick succession. The tubes were kept for 30 min. at 30^{o}C and the optical density (OD) of the colour developed was measured at 490 nm against the blank. D-Glucose was used as a standard and carbohydrate content was calculated by using the following formula.

$$\text{Carbohydrate \%} = \frac{\text{Standard value x OD of the sample}}{\text{weight of the sample}} \times 100$$

Estimation of DNA

The colorimetric method of Sadasivam and Manikam (1996) was adapted for the estimation of DNA on basis of quantitative reaction of deoxy sugar with diphenylamine reagent. 0.5 mg of dried yeast cells were thoroughly mixed with 1ml of saline citrate and made up to 3 ml with distilled water, to which 6 ml of diphenyalamine reagent was added. After mixing, the tubes were kept for 10 min. in boiling water bath and then cooled. The optical density (OD) of the colour developed was measured at 600nm against the blank. Known DNA was used the standard and DNA in samples was calculated by using this formula.

$$\text{DNA\%} = \frac{\text{Standard value x OD of the sample}}{\text{weight of the sample}} \times 100$$

Estimation of RNA

The colorimetric method of Sadasivam and Manikam (1996) was adapted for the estimation of RNA on the basis of pentose determination as well as orcinol, phloroglucinol, aniline etc. 50 µg of dried yeast cells were throughly mixed with 1 ml of distilled water and kept in ice chilled 10mM Tris-acetate, 1mM EDTA buffer (pH 7.2), and made up to 3 ml with distilled water. To this 6 ml of orcinol acid reagent and 0.4 ml of 6% alcoholic orcinol were added. After mixing, the tubes were kept for 20 min. in boiling water bath and then cooled. The optical density (OD) of the colour developed was measured at 660 nm against the blank. Known RNA was used the standard % of RNA of unknown samples were calculated as per the formula given below.

$$\text{RNA\%} = \frac{\text{Standard value x OD of the sample}}{\text{weight of the sample}} \times 100$$

Results and Discussion

SCP is normally considered as a source of protein. SCP may be used directly as human food supplement or it may be used in animal to at least partially replace the currently used protein-rich soybean meal and fish proteins and even cereals, which can be diverted for human consumption (Singh, 1998).The impetus behind single cell protein production lies partly in the need for more protein and partly in the commercial increase in the economic advantages gained by substitution of microbial protein for the conventional protein supplements used in livestock feeding (Khan *et al.*, 1992).

Microorganisms have the ability to upgrade low protein organic material to high protein food, and this had been exploited by industries. Yeasts are a polyphyletic group of Basidiomycetous and Ascomycetous fungi with an unifying characteristic of a unicellular growth stage. There are approximately 100 genera and 800 described species of yeasts. Their environmental role is similar to many other fungi, acting as saprophytes by converting plant and animal organics into yeast biomass and by-products, which may have commercial importance (Kurtzman and Fell, 1998). In general, yeast cells can grow very rapidly, the cell density can reach over 10^8 cells mL^{-1}, and the fermentation period seems to be short.

In the present study a total of 23 morphologically distinct yeast strains were isolated from the sediment samples. The potent isolate from sediment sample PMS9 was selected for further study and it was identified as *Saccharomyces cerevisiae* based on the biochemical tests (Table 1).

Table 1: Biochemical identification of *S. cerevisiae*

Test	Result
Gram reaction	+
Shape	oval, spherical
Colony	creamy smooth surfaces
Glucose	+
Glyceol	-
Calcium 2 – Ketogluconate	-
Arabinose	-
Xylose	-
Adonitol	-
Xylitol	-
Galactose	+
Inositol	-
Sorbitol	-
Methyl α –D- glucopyranoside	+
N-acetylglucosamine	-
Cellobiose	-
Lactose	-
Maltose	+
Saccharose	+
Trehalose	+
Melezitose	-
Raffinose	+

The yeast species that produce SCP include *Candida* spp., *Hansenula* spp., *Pitchia* spp., *Torulopsis* spp. and *Saccharomyces* spp. and are popular due to their high protein (Ravindra, 2000). Moreover, mainly *Saccharomyces cerevisiae*, have been used for centuries in food production. Furthermore, there is extensive information about their safety; they are

considered as Generally Recognized As Safe (GRAS)by the American Food and Drug Administration (FDA). Today, the only species fully acceptable as food for humans is *S.cerevisiae* (baker's and brewer's yeasts). In this regard, the present study included one more strain of this species as potential SCP.

In this study pH ranging from 4 to 9 was studied for the detection of optimum pH for the growth of *S. cerevisiae* in the SG broth. The maximum growth was noted at pH-6 (Fig - 1). Mitra *et al.*, 2012 found pH – 6 ideal for SCP production from *Saccharomyces cerevisiae*. Rajoka *et al.*, 2005 and Munawar *et al.*, 2010 produced maximum biomass of *Candida utilis* at pH 6.0.

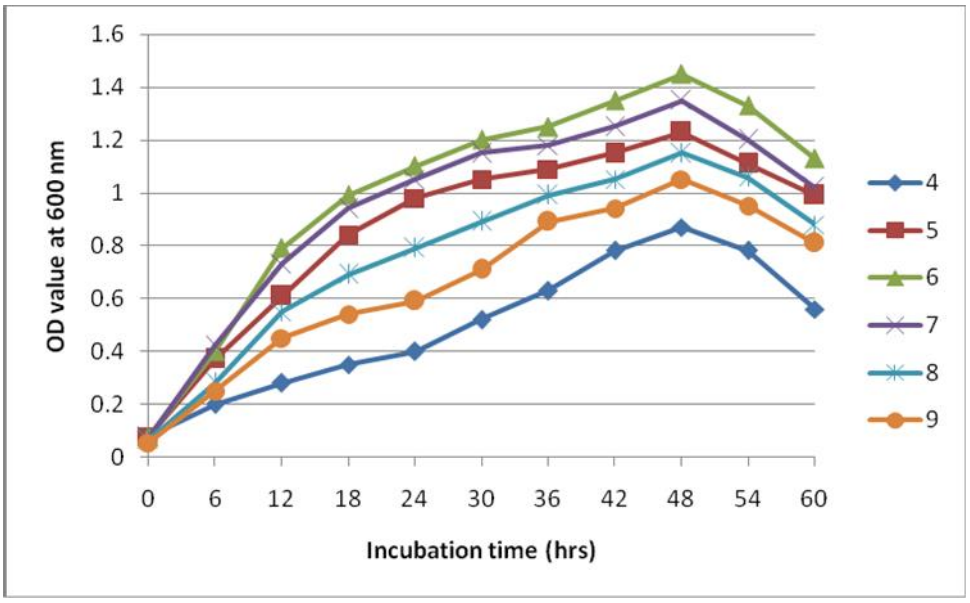

Fig. 1: Effect of pH on growth of *S. cerevisiae*

The temperature ranging from 25 to 40°C were studied for the detection of optimum temperature for the growth of *S. cerevisiae* in the medium. The maximum growth was noted at 30°C and minimum growth was observed at 25°C (Fig-2). Mitra *et al.*, 2012 observed 28°C ideal for SCP production from *S. cerevisiae*. Ojokoh and Uzeh (2005) reported 25°C ideal temperature for the maximum protein production in *S. cerevisiae*. Yalemtesfa *et al.*, 2010 found maximum protein content with *Chaetomium* sp and *A.niger* at 25°C.

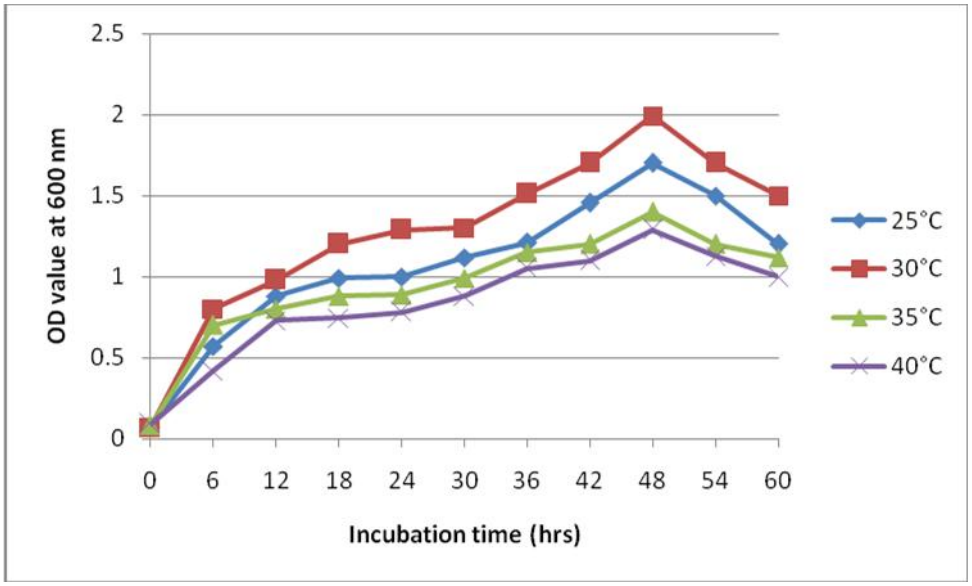

Fig. 2: Effect of temperature on growth of *S. cerevisiae*

The salinity ranging from 0 to 4% were studied for the detection of optimum salinity for the growth of *S. cerevisiae* in the medium. Regarding NaCl concentration on growth, the maximum growth was noted at 0% NaCl (Fig-3). The different incubation period from 0 to 60 hrs were studied for the detection of optimum incubation period for the growth of *S. cerevisiae* in the medium. The maximum growth was noted at 48 hrs (Fig-4). Munawar *et al.*, 2010 reported 96 hrs whereas Li *et al.*, 2009 optimized fermentation period of 69 hrs was best maximum cell biomass production of *Candida utilis*.

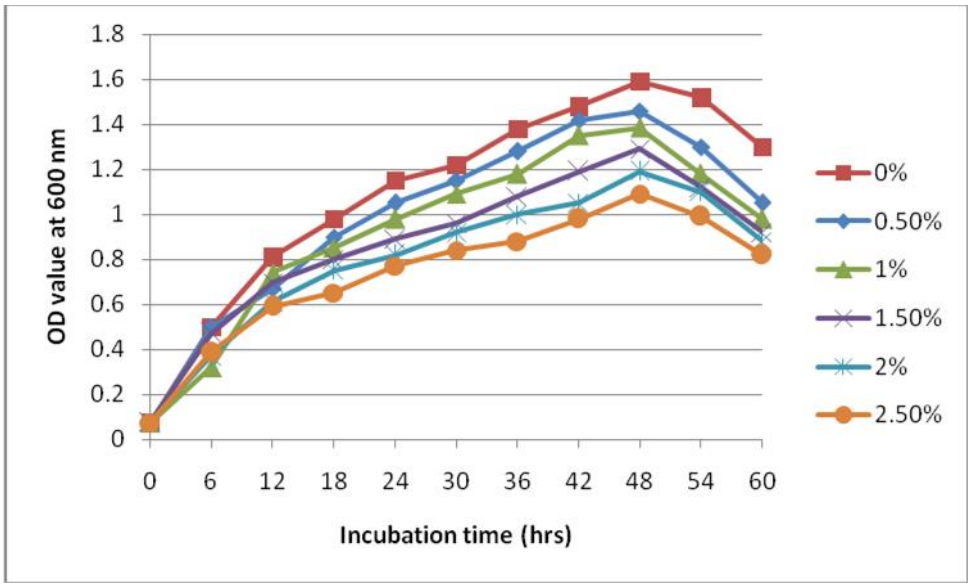

Fig. 3: Effect of salinity on growth of *S. cerevisiae*

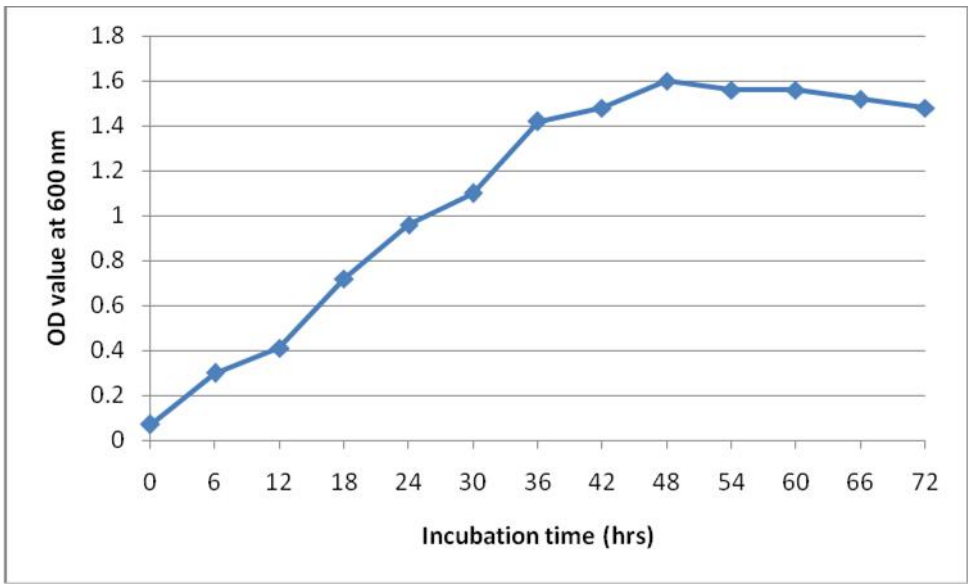

Fig. 4: Effect of incubation period on growth of *S. cerevisiae*

When glucose, maltose, fructose, sucrose and starch at 5% concentration were tested, maximum growth was observed with sucrose (Fig-5). When the concentration of ideal carbon source (i.e) sucrose was tested from 1 to 5%, 3% resulted in maximum growth (Fig-6). Ojokoh and Uzeh (2005) reported 35.5% protein production in *S. cerevisiae* biomass when using 2% glucose in papaya extract medium. Mitra *et al.*, 2012 found 0.6% cassava media as a carbon source ideal for SCP production from this species.

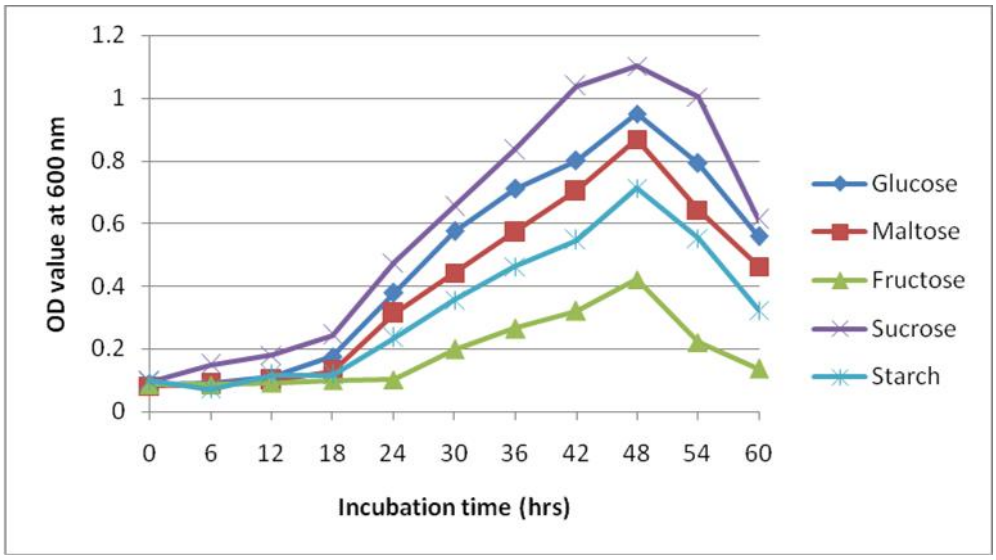

Fig. 5: Effect of carbon source on growth of *S. cerevisiae*

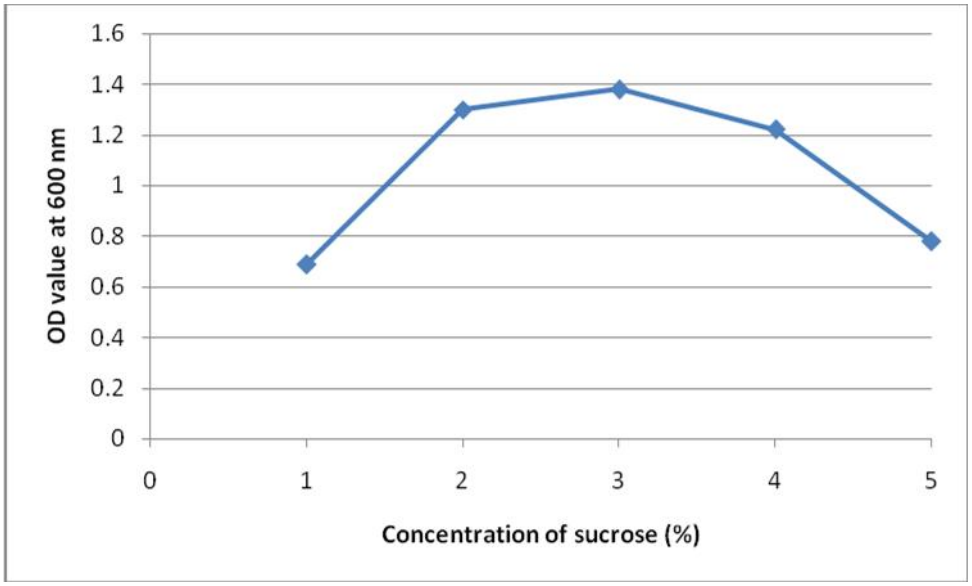

Fig. 6: Effect of concentration of carbon source on growth of *S. cerevisiae*

When 0.5% yeast extract, beef extract, peptone, ammonium nitrate, ammonium sulphate and potassium nitrate were examined, yeast extract as a nitrogen source exhibited the maximum growth (Fig-7). When yeast extract was tested at varying concentration (i.e) 0.1 – 1.0%, 0.5% resulted in the maximum growth (Fig-8). Ojokoh and Uzeh (2005) reported 35.5% protein production in *S. cerevisiae* biomass when using 0.25% $(NH_4)2HPO_4$ in papaya extract medium. Mitra *et al.*, 2012 found 0.82% yeast extract as a carbon source ideal for SCP production from the same species.

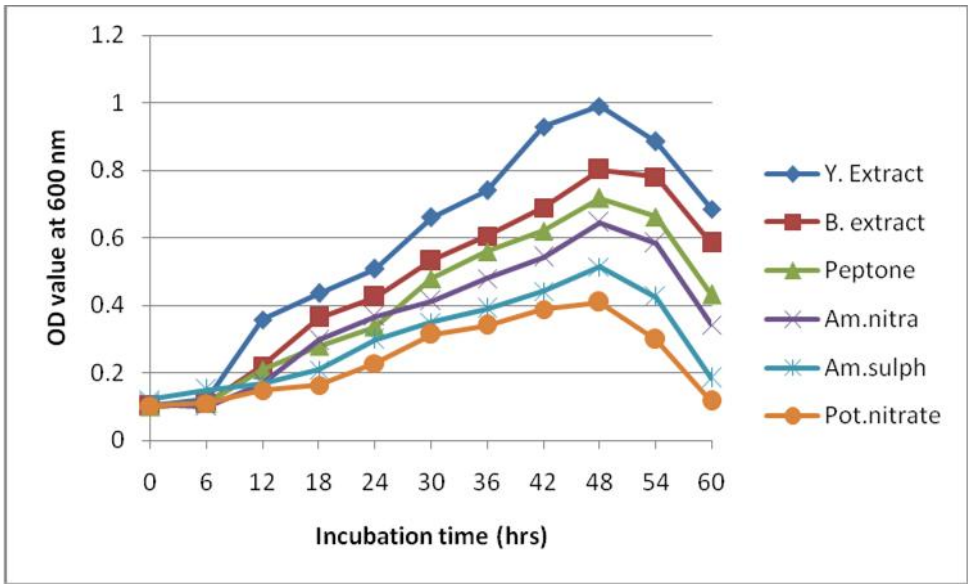

Fig. 7: Effect of nitrogen source on growth of *S. cerevisiae*

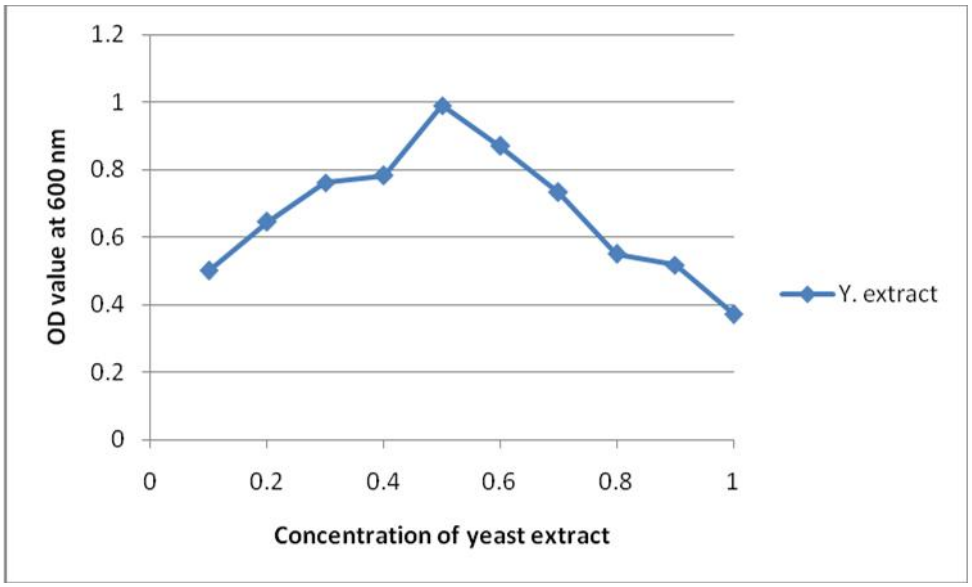

Fig. 8: Effect of concentration of nitrogen source on growth of *S. cerevisiae*

The potential strain identified as *Saccharomyces cerevisiae* was kept for mass scale culture with the optimized growth parameters such as pH-6, 30^{o}C 0% NaCl, 48 hrs incubation, sucrose as carbon source (3%), and 0.5% yeast extract as nitrogen source. After incubation harvest was done and cells were separated by centrifugation at 10, 000 rpm for 30 min. The weight of biomass was gravimetrically done and expressed as on dry weight basis g/L.

In this study the biochemical composition of *S. cerevisiae* biomass is presented in Table 2. The total biomass yield was 5.14g/L. Crude protein, total carbohydrate and total nucleic acid content of biomass were respectively 40.5%, 30%, and 5.8% before nucleic acid reduction whereas after nucleic acid reduction they were found be 44.5%, 30% and 1% respectively (Table 2). Chandra and Chakrabarti (1996) reported 45.6% of SCP crude protein content production by *Saccharomyces cerevisiae* yeast using plant liquid waste.

Maragatham and Panneerselvam (2011) obtained 34% of crude protein using papaya fruit as substrate for single cell protein production from this species. Ojokoh and Uzeh (2005) reported 35.5% protein production in the same species using biomass in papaya extract medium. The present study showed *S. cerevisiae* of estuarine origin can be used as SCP.

Table 2: The composition of lyophilized *S. cerevisiae* biomass grown on optimized medium

Composition	Before nucleic acid reduction (%)	After nucleic acid reduction (%)	Standard values of Food Grade Yeast
Crude protein	40.5	44.5	35.7
Total Carbohydrate	30.0	30.0	46.3
Total Nucleic acid	5.8	1.0	Below 3
RNA	4.0	0.6	
DNA	1.8	0.5	

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