

## **Process optimization for the production of Yeast Extract using fresh Baker's yeast**

**Muhammad Asjad Khan**

Department of Biotechnology  
Virtual University of Pakistan Lahore.  
Email: [asjad.khan@vu.edu.pk](mailto:asjad.khan@vu.edu.pk)

**Muhammad Mohsin Javed**

Department of Biotechnology, Virtual University of Pakistan

**Asia Ahmed**

Department of Molecular Biology, Virtual University of Pakistan

**Sana Zahoor**

Department of Biotechnology, Virtual University of Pakistan

**Kaleem Iqbal**

Human Cancer Genomics Research, King Faisal Hospital and Research Center, Riyadh KSA

### **Abstract:**

*Yeast extract is extensively applied in various food industries as a food additive to enhance to flavor of food products or as a vitamin supplement. It is also considered as a crucial component of microbiological media. The current study was conducted to optimize a process for the production of yeast extract by using Baker's yeast (*Saccharomyces cerevisiae*). The cultivation of yeast biomass was performed in a stirred fermenter. The influence of numerous physical and chemical parameters such as carbon and nitrogen sources, temperature, pH and agitation were evaluated on the production of yeast cells by employing one factor at a time approach and optimum conditions for the production of maximum yeast biomass was determined. The maximum growth was attained using molasses as a substrate at 30°C supplemented with urea at 150 rpm with pH 4.5. After fermentation, cells were separated by centrifugation and were ruptured by adopting different techniques and autolysis was found the most viable method. Various techniques were applied to dry the yeast extract and the spray dryer was appeared as most effective one. Yeast extract acquired after drying was subjected to various analysis including protein and solid content estimation and amino acid profiling and compared with commercial yeast extract. The dried yeast extract was incorporated in media preparations to grow various microorganisms including yeast,*

*fungi and bacteria and considerable growth was observed. These promising results indicated that the developed process is a cost effective alternative approach for the production of yeast extract.*

## Keywords

Yeast extract, Fermentation, *Saccharomyces cerevisiae*, Food Microbiology

## 1. Introduction

Yeast, *Saccharomyces cerevisiae*, is ubiquitously present in the environment and has great applications in various industries especially in the production of alcoholic beverages and to leaven the bread dough [Banat & Marchant, 1995; Russell & Stewart, 1998; Walker, 1998]. Yeast extract, obtained from the lysis of yeast cells, mainly contains amino acids, vitamins, nucleotides, peptides, carbohydrate, salts and other water soluble components of yeast cells. However, nitrogenous component and vitamins are the vital ingredients of the yeast extracts because of their nutritious properties. It is widely used in food industries as a flavouring agent in sauces, soups, stews, gravies, canned and snack foods. It also possess extensive applications in health sector as a vitamin and protein supplement as well as in microbiological media preparations, in cosmetic industry and for plant nutrient [Tanguler & Erten, 2008].

Yeast extracts commercially available in various forms such a powder, paste or liquid [Nagodawithana, 1992; Sommer, 1998] and mainly produced by the baker's or spent brewer's yeast, *Saccharomyces cerevisiae*, by autolysis. Though, other yeasts including *Candida utilis* and *Kluyvermyces marxianus* are also used [Sommer, 1998]. Since the brewer's yeast is served as inexpensive source of yeast extract production for food and fermentation industries-[Stam et al., 1998], however, yeast extract extracted from brewer's yeast has very undesirable bitter taste due to the absorption of hop constituents and beer solids to the yeast cells during beer fermentation [Sombutyanuchit et al., 2001]. Therefore, de-bittering is required for such yeast creams to reduce the bitter taste in final product [Shotipruk et al., 2005]. Despite this limitation, brewers yeasts have been applied by various researchers for the production of yeast extract [Tanguler & Erten, 2008]. Similarly, baker's yeast has also been investigated by several biotechnologists for the production of yeast extract [Vukašinović-Milić et al., 2007; Zarei et al., 2017]. This study was aimed to develop a process of yeast extract production from baker's yeast.

## 2. MATERIALS AND METHODS

### 2.1 Organism and inoculum preparation

Yeast (*Saccharomyces cerevisiae*) was purchased from Sigma-Aldrich (CAS-No: 8013-01-2) and maintained on Yeast Peptone Dextrose (YPD) agar containing 3.0 g yeast extract, 5.0 g peptone, 10.0 g Dextrose and 20.0g agar (pH 5.5) at 4 °C. The inoculum was prepared by transferring a single colony to 250 mL Erlenmeyer flask containing 50 mL YPD broth medium and incubated at 30°C at 200 rpm in a rotary shaker for 24 h.

### 2.2 Optimization of Fermenter production of yeast biomass

Stirred tank fermenter (7.5 L) was used for the production of yeast cells. Different factors such as media (yeast extract, potato dextrose, sabouraud dextrose broth, yeast extract peptone dextrose plus adenine, molasses, whey), nitrogen sources (urea, peptone, yeast extract, NH<sub>4</sub>Cl, Na<sub>2</sub>NO<sub>3</sub>), temperature (20-40°C), pH (3-6) and agitation (50-200 rpm) were evaluated for their optimum levels for the maximum production of yeast biomass. Five liter fermentation media with 25% inoculum was used for each fermentation batch. The fermenter vessel was sterilized under 121°C temperature for 15 min. Sterilized solution of 1N NaOH and 1N

HCl was used for pH control, whereas silicon oil was used to control foaming during fermentation. The fermentation was conducted for a period of 12 h for each batch unless mentioned.

### **2.3 Removal of water from yeast cell biomass**

After optimum production of yeast biomass, the whole content from the fermenter was transferred to the rotary vacuum filter in order to remove the water from wet yeast cells and the yeast cells were obtained in the form of small cakes. These cakes were then further processed for cell lysis in next step.

### **2.4 Cells disruption/lysis**

Cell lysis is a significant step during the production of yeast extract as it contributes the overall cost of the final product. Different techniques were adopted by keeping in view the financial feasibility. These methods were sonication, use of liquid nitrogen, acetone and toluene, bead mill and autolysis [Liu et al., 2016; Tanguler & Erten, 2008]. Results were recorded in term of Brix. After the rupturing of the cells, the whole material were subjected to centrifugation (Avanti J-26 XP centrifuge) for 10 min at 6000 rpm at 10 °C and supernatant was collected and stored at 4 °C till further use.

### **2.5 Final Drying**

Various methods were screened to dry the yeast extract obtained after separation of cell debris. These methods including Rotary vacuum filter, Spray dryer (Spray Dryer SD-05), Rolling bed filter, Fluidize Bed dryer and Pneumatic dryers.

### **2.6 Chemical analysis and comparison of produced yeast extract with commercial yeast extract**

After getting the product in its final crystalline powder form, some compositional tests were performed. For the estimation of total solid content, hand refractometer was employed and the total solid content was measured in term of brix by placing one drop of supernatant at hand refractometer [Tsai et al., 2018]. Protein content was measured by using Bradford protein assay [Bradford, 1976]. The presence of amino acids was investigated by thin layer chromatography (TLC) technique in both commercial and produced yeast extract by using the previously reported method [Brenner and Niederwieser, 1967].

### **2.7 Assessment of microbial growth using prepared yeast extract**

An experiment was performed to compare the biological activity of prepared yeast extract vs. different yeast extracts available in market. Growth media was prepared by dissolving malt extract (2g), agar (2g) and prepared or commercial yeast extract (2g) in 100 mL of distilled water. Media was inoculated with *Geotrichum candidum*, *Aspergillus niger*, *Escherichia coli* and *Bacillus subtilis* separately and incubated at 30 °C for fungi or at 37 °C in case of bacteria for 24 h.

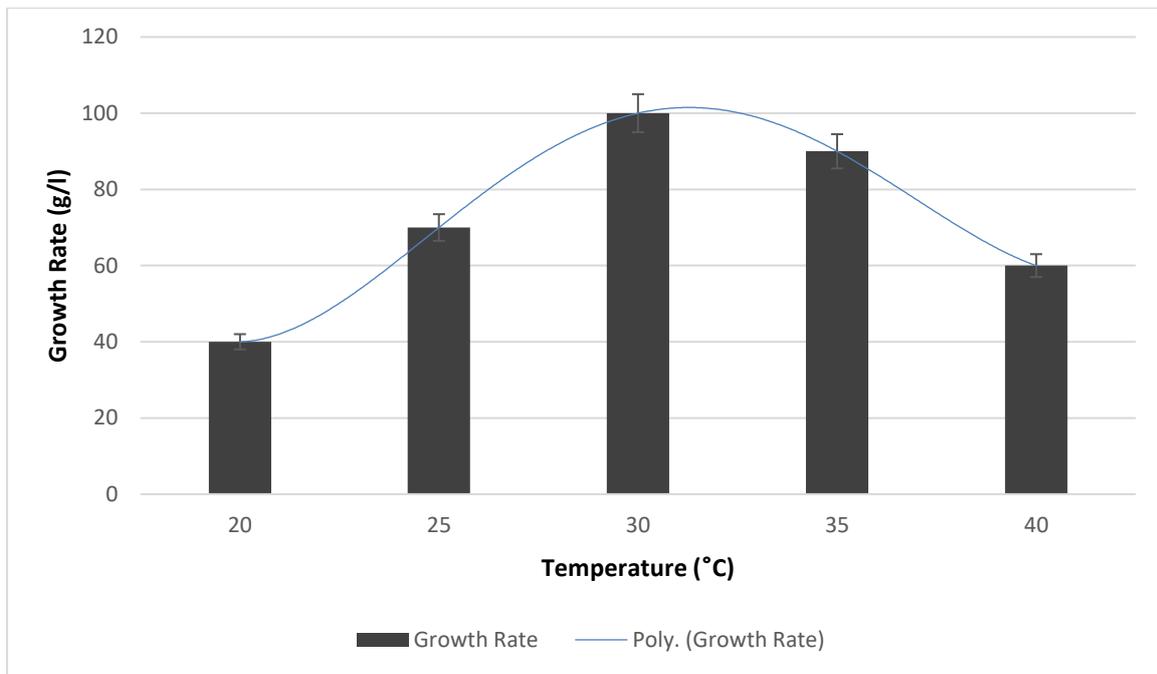
## **3. RESULTS AND DISCUSSION**

Yeast extract is extensively used in different industrial sectors as a flavouring agent or nutrient supplement. In this study, a process was designed and optimized for the production of yeast cells (*Saccharomyces cerevisiae*). These cells were processed further for the extraction of yeast extract.

### **3.1 Optimization of production of maximum yeast biomass**

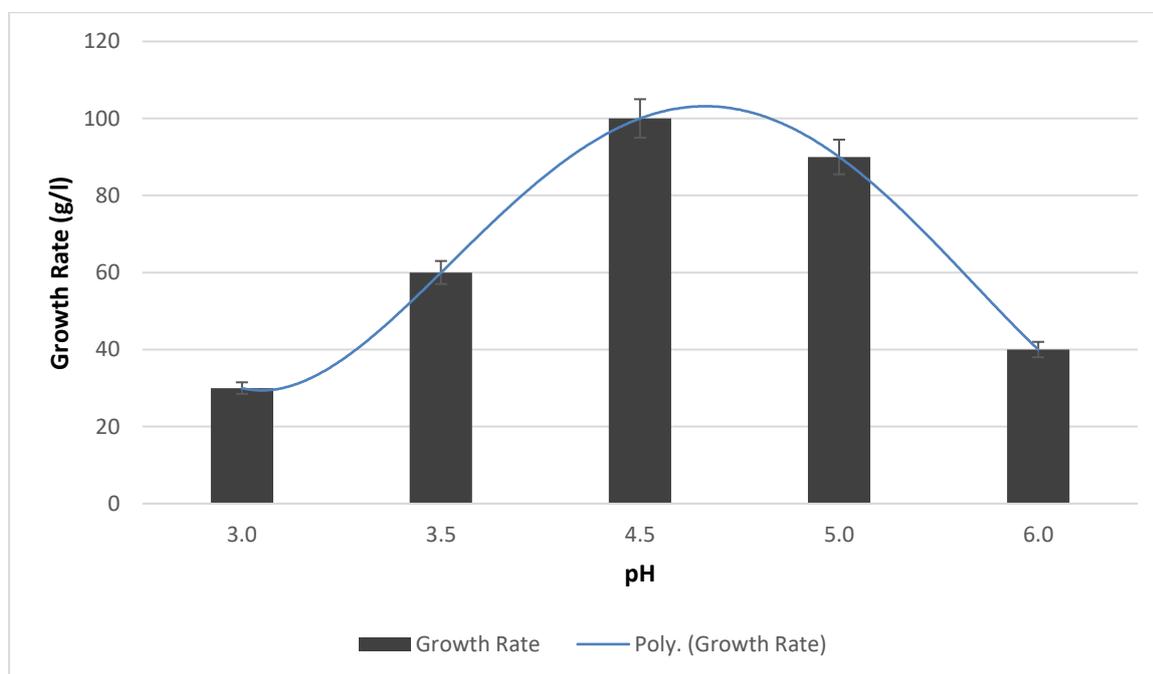
There are several physical, chemical and biological factors that affect the growth of microorganism. These parameters were optimised by adopting one factor at a time strategy for maximum yeast biomass production.

Temperature is a significant factor that plays a vital role in the growth of microorganism. Yet, each organism requires a specific temperature for their growth [Darah et al., 2013]. Maximum yeast biomass production was obtained at 30 °C (Figure 1). The optimum temperature for the growth was in concordance with the results reported previously for the growth of *Saccharomyces cerevisiae* using cane molasses as a substrate [Ghorbani & Younesi, 2013; Walsh & Martin, 1977; Win et al., 1996].



**Figure 1:** Effect of different temperatures on the growth rate of yeast cells: X-axis represents temperature in degree Celsius, Y-axis represents growth rate in gram/liter

The pH is also a vital factor regarding the growth of microorganisms. It has been reported that pH play an important role in the ionization of enzyme and transport of nutrients across the cell membrane, hence, maintaining the pH at optimal level is critical to ascertain the maximum growth. Out of different selected pH values used for the growth of yeast biomass, the maximum growth was obtained at pH 4.5 (Figure 2). It has been reported that maximum growth of *S. cerevisiae* was obtained at a pH range of 4.5 - 5.5 [Ghorbani & Younesi, 2013; Thomas et al., 2002].



**Figure 2:** Effect of various pH on the growth rate of yeast cells: X-axis represents pH, Y-axis represents growth rate in gram/liter

When the effect of different carbon sources was determined on the growth of yeast cells by culturing them using different carbon sources, the highest growth yield was obtained with molasses (100 g/L) as compared to other screened media (Table 1). Previously, several researchers reported the maximum production of *S. cerevisiae* using cane molasses as a substrate [Ghorbani & Younesi, 2013; Wang et al., 1979].

**Table 1:** Growth yield of yeast biomass by using different carbon sources

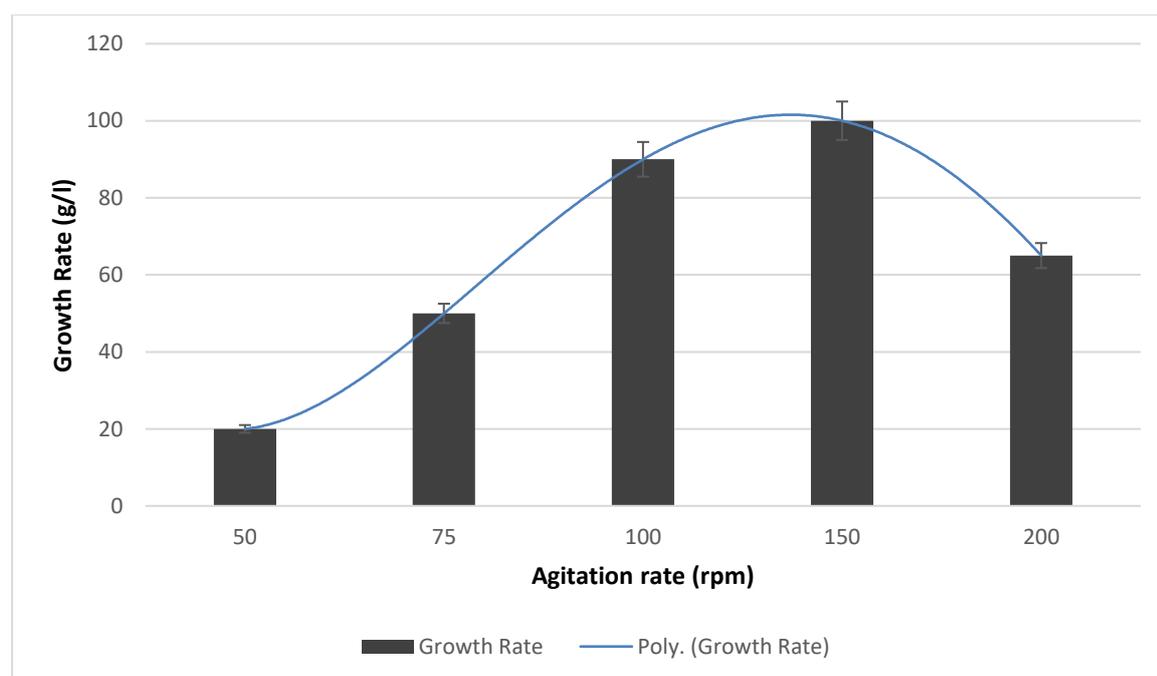
S. no.	Media	Growth yield (g/L)
1	YPD	70
2	SDB	40
3	YPAD	60
4	Molasses	100
5	Whey	50

The effects of different nitrogen sources i.e., yeast extract, urea,  $\text{NH}_4\text{Cl}$  and  $\text{Na}_2\text{NO}_3$  was also determined on the production of biomass (*Saccharomyces cerevisiae*). It was observed that the growth of biomass was optimum when yeast extract was supplemented as nitrogen source (Table 2). It has been reported

that *S. cerevisiae* is able to utilize a wide variety of nitrogen sources including organic and inorganic, nevertheless, various nitrogen sources were found optimum for the growth of *S. cerevisiae* including urea [Yue et al., 2012] and yeast extract [Laopaiboon et al., 2009] previously. The maximum growth was obtained at 150 rpm as shown in figure 3, commonly used for the growth of *S. cerevisiae* [Li et al., 2017].

**Table 2:** Effect of different nitrogen sources on biomass production

S. no.	Nitrogen Sources	Biomass Production (g/L)
1	Yeast Extract	12.00
2	Peptone	10.00
3	Urea	0.5 0
4	NH <sub>4</sub> Cl	1.00
5	Na <sub>2</sub> NO <sub>3</sub>	1.00



**Figure 3:** Effect of agitation on the growth rate of yeast cells: X-axis represents agitation rate (rpm), Y-axis represents growth rate in gram/liter

### 3.2 Cell rupturing process

After fermentation, the whole content from fermenter was subjected to rotary vacuum filter to obtain yeast biomass cakes. Following this, different strategies were opted for the lysis of yeast cells. Autolysis was

observed as a most efficient method with highest value of brix (Table 3). This result is consistent with the reports of others [Conway et al., 2001].

**Table 3:** Effect of different methods on the lysis of yeast cells

S. No	Process	Brix (Bx)
1.	Use of Acetone and Toluene	15
2.	Sonication	13
3.	Pastel and mortar	11
4.	Liquid Nitrogen	17
5.	Autolysis	19

### 3.3 Drying

The soluble content was subjected to drying by employing different methods. All procedures were analysed and it was found that spray dryer gives best results (Table 4). Hence, spray dryer method is used extensively for the crystallization of yeast extract. [Tanguler & Erten, 2008].

**Table 4:** Different methods used for the drying of produced yeast extract

S. No.	Methods of drying	Flow rate of liquid (L/h)
1	Rotary vacuum filter	0.1
2	Spray dryer	0.2
3	Rolling bed filter	1.5
4	Fluidize Bed dryer	0.9
5	Pneumatic dryers	1.7

### 3.4 Chemical analysis and comparison of produced yeast extract with commercial available yeast extracts

Total solid content was determined by hand refractometer and both commercial and produced yeast showed 5 Brix. Protein content in both the yeast extracts was estimated by Bradford's method and both were found to have 13.75 and 13.5 mg/mL of proteins, respectively. TLC is one of cheapest and reliable method for quantitative and qualitative analysis of amino acid. The results of TLC showed that commercial and produced yeast extract have the same number of amino acids (Figure 4). The chromatogram also depicted that the produced yeast extract possessed elevated concentrations of amino acids as compare to commercial yeast indicating the improved quality of prepared yeast extract. The amino acids were identified as leucine, isoleucine, histidine, aspartic acid, arginine, lysine, and glutamine.



**Figure 4:** Amino acid profiling of produced and commercial yeast extracts by Thin Liquid Chromatography (TLC) plate. Rf values: Leu: 0.31, Ile: 0.30, His: 0.66, Asp: 0.72, Arg: 0.28, Lys: 0.57, Gln: 0.68.

### 3.5 Assessment of Biological activity

The growth of different bacteria and fungi was evaluated in the media prepared by produced yeast extract. Considerable growth was observed in the slants with prepared yeast extract that was comparable with the growth of organisms in the media with commercial yeast extracts. In addition, the organisms showed late growth in commercially prepared media (After 24 h) whereas the early growth (within 16-24) was obtained in the media with produced one indicated that the produced yeast extract is a cost-effective alternate to be applied in the preparation of microbiological media.

## 4. CONCLUSIONS

The developed process for the production yeast extract have several advantages such as no chemicals or enzymes were added during extraction process which resulted in the reduction of cost and number of steps in down streaming processes. The chemical analysis showed that prepared yeast extract was comparable with commercial one in term of its properties. In the end, application of produced yeast extract in microbiological media revealed its suitability for the growth of different microorganisms. With these promising results, this process could be an efficient alternate for the lab or commercial scale production of yeast extract specifically for microbial growth.

### Conflict of interest

The authors declare there are no conflicts of interest.

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