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Investigation of the Best *Saccharomyces cerevisiae* Growth Condition

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Abstract

Introduction: *Saccharomyces cerevisiae* is known as one of the useful yeasts which are utilized in baking and other industries. It can be easily cultured at an economic price. Today the introduction of safe and efficient carriers is being considered. Due to its generally round shape, and the volume that is enclosed by its membrane and cell wall, it is used to encapsulate active materials to protect them from degradation or to introduce a sustained release drug delivery system. Providing the best conditions in order to achieve the best morphological properties of *Saccharomyces cerevisiae* as a carrier.

Methods: In this research, the most suitable growth condition of yeast cells which provides the best size for use as drug carriers was found by a bioreactor in a synthetic culture medium. Yeast cell reproduction and growth curves were obtained, based on pour plate colony counting data and UV/Visible sample absorption at 600 nm. Yeast cell growth patterns and growth rates were determined by Matlab mathematical software.

Results: Results showed that pH=4 and dissolving oxygen (DO) 5% was the best condition for yeast cells to grow and reproduce. This condition also provided the largest size (2 × 3 μ) yeast cells.

Conclusion: Owing to the yeast cells' low-cost production and their structural characteristics, they could be used as potent drug carriers.

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Keywords: Carrier, Growth, Mathematics, *Saccharomyces cerevisiae* yeast cells

1. Introduction

The name *Saccharomyces cerevisiae* is composed of two parts: The first part "Saccharo" means "sugar fungus" in Latinized Greek while the second part "cerevisiae" means "of beer". *S. cerevisiae* is known as one of the useful yeasts which are utilized in baking and other industries. It is used as a eukaryotic model organism in biological studies, because it can easily be cultured. This organism carries out the most common type of fermentation. It has a round to ovoid shape and it reproduces by a budding mechanism (1, 2). The yeast cells can grow in haploid and diploid forms. Haploid cells indicate a simple mitosis life cycle that, under stressful conditions will die. Diploid cells, like the haploid ones, show the mitosis life cycle, but under high stress situations, enter the meiosis life cycle and produce four haploid spores. Their doubling time is approximately 90 min (3-6). *S. cerevisiae* can grow aerobically and anaerobically. Its ability to use different sugars depends on which way it grows. If it grows aerobically, galactose and fructose are the best fermenting sugars. All strains require nitrogen and phosphorus sources to grow. To prepare nitrogen, they consume ammonia and urea. They use dihydrogen phosphate as a source of phosphorus. They also need sulfur and various metals such as magnesium for optimum growth. Due to gender differentiation, yeast cells have two mating types: a, and α. Two haploid yeast cells of different types can mate with each other. In this situation mating leads to genetic recombination. Almost all the yeasts have buds. As the cells

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grow, the buds grow too until they become mature. They then separate and leave their parents (7). The *S. cerevisiae* yeast cell can be employed as an efficient carrier due to its food-grade and low-cost characteristics. *S. cerevisiae* is made up of an external thick cell wall which comprises a beta-glucan network and a small amount of chitin associated with a mannoprotein layer. These cell wall properties cause a yeast cell to be a sustained release type carrier. The mechanical strength of the yeasts' structures allows them to load various active materials (8-10). Several experiments in various conditions were carried out in order to improve yeast cell numbers and sizes for use as carriers. Many growth curves have been applied to model biological growth. The logistic growth equation is the most known and efficient model. Different extended growth equations originate from this model according to its restrictions and limitations. The Richard growth equation is one of the extended models which showed the highest correlation with our data (11-15).

2. Material and Methods

2.1. Culture of yeast cells

S. cerevisiae was provided by the industrial research organization, Tehran, PTCC 5269. Bioreactor (Winpact, FS-01-A series (double jacketed vessel)) was used to culture the yeast cells. Bioreactor was applied, to establish the best environmental conditions for the yeasts to grow. It was composed of three parts: controller, gas mixer and gas analyzer. The bioreactor vessel (3 liters) was filled by 2 liters synthetic culture medium (10 g KH_2PO_4, 4 g $(NH_4)_2SO_4$, 0.8 g $MgSO_4$, 2 g yeast extract, 10 g glucose) (Merck). Three ml Suspension of yeast cells (10^8 cfu/ml) in physiologic serum was added to the vessel. The temperature was set at 30 °C and the culture medium was stirred by the rate of 200 rpm. The bioreactor contains a double jacket vessel. The water which circulates around the vessel, between the two jackets, helps the culture medium to maintain its temperature. The temperature of water which circulates around the jacket should be 10–15 °C lower than the temperature of synthetic culture medium. Previously, growth of *S. cerevisiae* was optimized (16). In the present research, the effects of two parameters of synthetic medium (pH and percentage of dissolving oxygen (DO)) in nine situations on *S. cerevisiae* growth rate were studied. Culture medium pH was adjusted to 4, 5 and 6 by HCl 1M and the percentage of DO was adjusted to 5, 10 and 15% by gas mixer.

2.2. Determination of yeast cell growth curves

Growth of the yeast cells in each condition was defined by colony counting of the fourth diluted plate by colony counter (acolyte (symbiosis)). One milliliter of culture medium was diluted about 10^{-1} times with physiologic serum four times and the dilutions were introduced to four separate TSA plates. In addition, UV/Visible (CECIL 9000) absorption of culture medium samples at 600 nm in 0 to 13 hours were obtained, to plot the common growth curve of microorganisms which were composed of lag, log, stationary and decline phases. Yeast cell growth patterns and growth rates were evaluated by Matlab mathematical software.

2.3. Determination of yeast cell size

Size of the yeast cell in the log phase was measured each hour for up to 10 hours by light microscopy and the mean size was determined. If the yeast cell is used as a carrier to load the active materials, its size should be appropriate enough.

2.4. SEM images

Scanning electron microscopy (Oxford Company, S-360) was used to study the morphological shape of yeast cells in pH=4 and DO 5%. The cells were embedded in paraffin, sectioned, de-paraffined, and sputter-coated with gold.

3. Results

3.1. Determination of yeast cell growth

Growth curves of the yeast cells were studied in nine situations (3 different amounts of pH × 3 different amounts of percentage DO) (Figures 1-2). Matlab mathematical software was used to design an equation for description of the growth pattern of the yeast cells based on colony counting (CFU/ml×10^8) (Y axis) against time (h) (X axis) in pH=4 and DO 5% which was the best condition. The yeast cell growth curve (pH=4 and DO5%) based on colony counting was fitted by the Richard equation to estimate the maximum growth rate (Figure 3). The Y and X axes showed maximum growth rate and time (h), respectively.

3.2. Determination of yeast cell size

Light microscopy was applied to measure the mean size of yeast cells in the pH=4 and DO 5% condition, (Figure 4):

3.3. SEM images

The SEM image of yeast cells in pH=4 and DO 5% condition is shown in Figure 5.

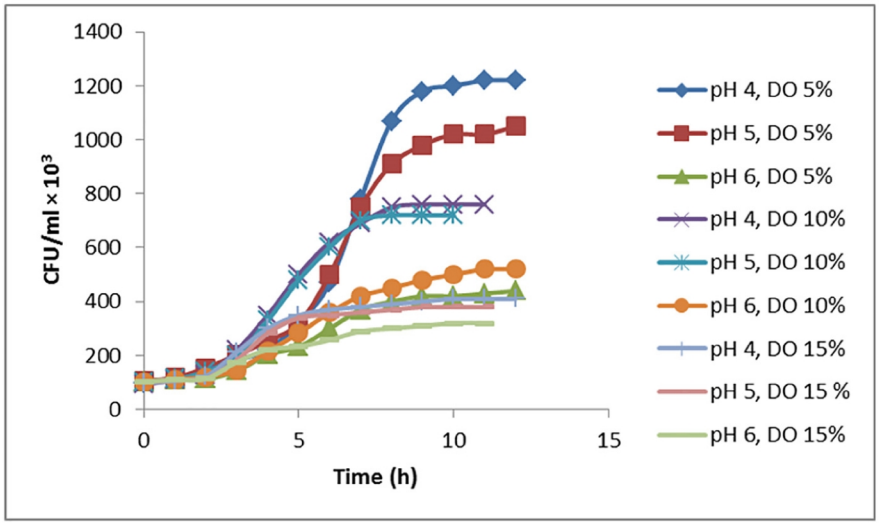

Figure 1. Growth curves of *Saccharomyces cerevisiae* based on colony counting

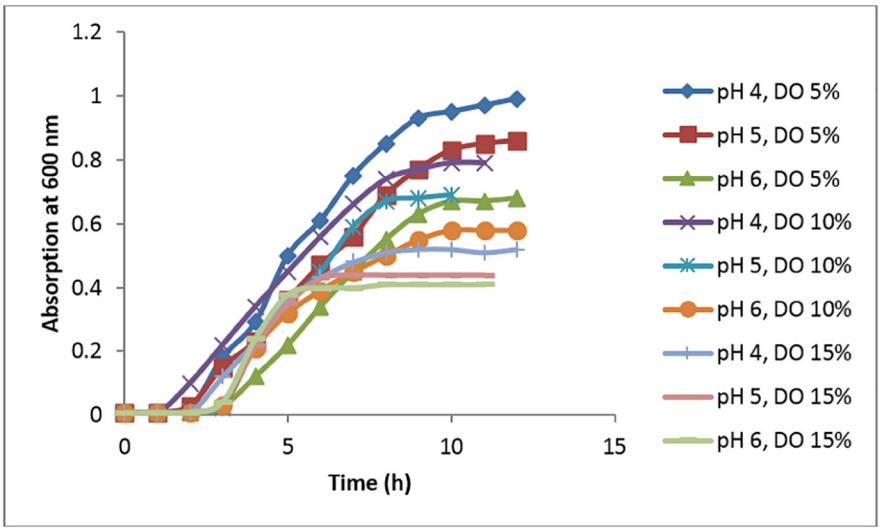

Figure 2. Growth curves of *Saccharomyces cerevisiae* based on UV/Visible absorptions

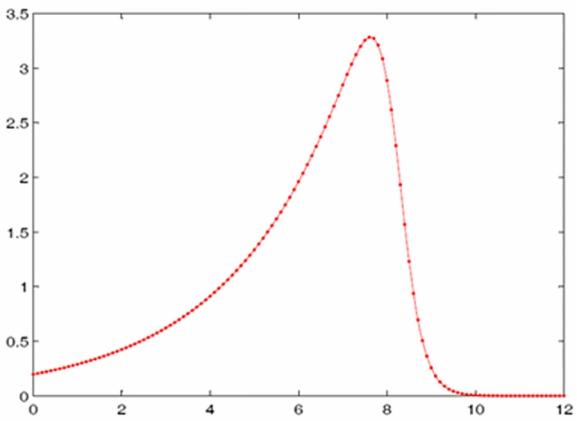

Figure 3. Growth rate curve of *Saccharomyces cerevisiae* in pH=4 and DO5%

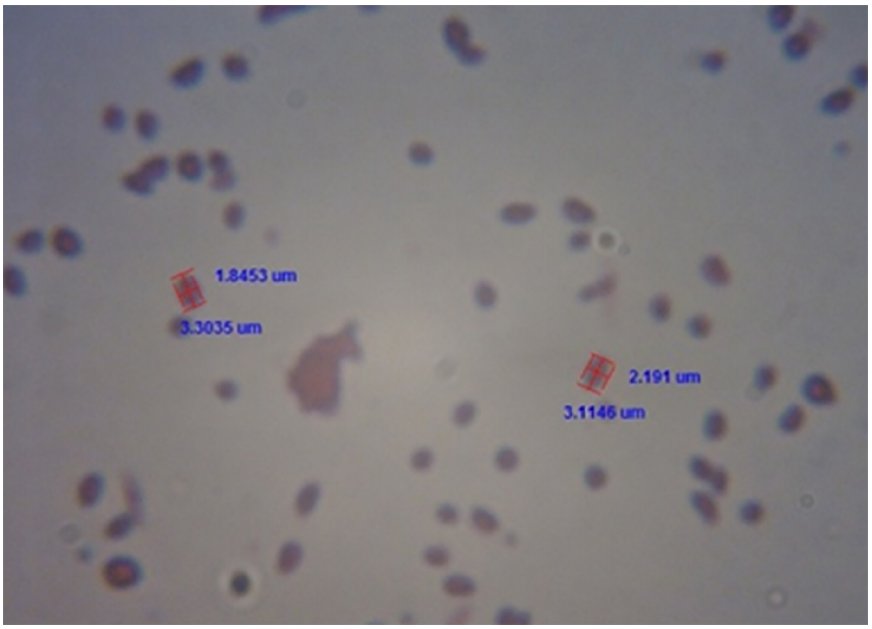

Figure 4. Light microscopy image of *Saccharomyces cerevisiae* in pH=4 and DO5%

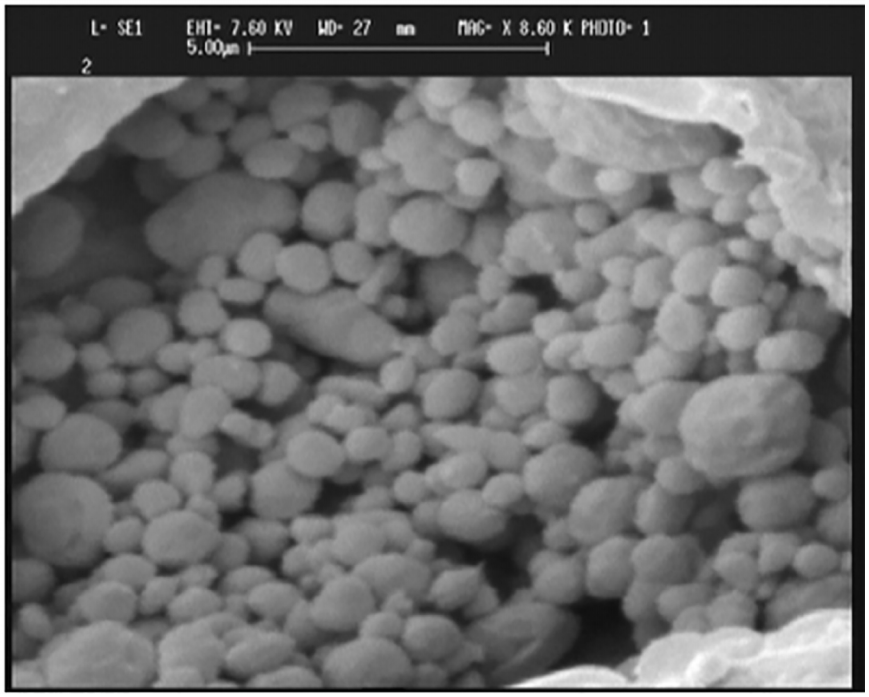

Figure 5. SEM image of *Saccharomyces cerevisiae* in pH=4 and DO 5%

4. Discussion

Today the introduction of safe and effective carriers is being considered. The carriers are highly desirable when they are natural, and cost little to produce. An efficient carrier should have a proper loading ability. Structurally, it should also have the ability to control the release of active ingredients well. *Saccharomyces cerevisiae* yeast cells as carriers have all the advantages mentioned above. With this vehicle, buying the expensive ingredients of a synthetic carrier is not necessary (10). By providing a suitable culture medium, millions of yeasts could be obtained to be used as carriers. The culture medium should prepare the best conditions for yeast growth to load the active materials in the best way. In order to provide suitable loading capacity and cell wall uniformity, growth of yeast cells was optimized. The pH and percentage DO are the two parameters which were optimized, to provide the best situation for yeast

overgrowth. Several mathematical equations and software were studied to explain the yeast's growth process. Among these equations and software, the Richard growth equation and Matlab mathematical software were appropriate for our data. Yeast cell growth in different conditions was studied (Figures 1-2). It was proved that pH=4 and DO 5% provided the best condition for yeast cells to reproduce and grow. This condition improved not only the yeast cells' reproduction and growth but also their size as carriers. In this situation, the growth curve showed a short lag phase (two hours). The log phase had the sharpest slope and lasted seven hours. At the end of log phase, the number of yeast cells reached 1.2 × 109 which was the maximum reproduction amount. UV/Visible absorptions at 600 nm also confirmed the results and it was at the maximum, about 1, at the end of the log phase. Comparisons of different parameters which affect yeast cell growth were investigated. The results showed that yeast cells could reproduce better in lower pH and percentage DO. In three different amounts of percentage DO, by decreasing the acidity, the growth cycle decreased. Better growth in higher acidity conditions may be due to the ethanol metabolite of yeast cells. Acid neutralized the ethanol produced by the yeast cells and allowed them to continue their growth. Higher percentage DO, in the same way as higher pH, prevents yeast cell growth due to ethanol oxidation, which is a potent preventive factor for growth (17-19). As shown in the curves, the growth was improved in DO by 10%, but when the results were fitted by Matlab software and the Richard equation, this misleading improvement was refused. Matlab software estimated the best equation to describe the growth pattern in every situation. In the first stage of the yeast cell growth cycle, absorptions were changed more than the numbers of yeast cells. In this situation, at first, the number of yeast cells was constant and only the yeasts and the buds originated from them continued growing. So the absorptions were increased without increase in the number of yeast cells. Among the various branches of generalized logistic equations, the Richard equation (Figure 3) was the equation best fitted to the results with a maximum correlation coefficient to calculate the maximum growth rate in each situation. The yeast cells reached the highest growth rate (3.309) in pH=4 and DO 5% condition. In this condition, the longest time of the growth phase (7.8 hours) was obtained. Yeast cell size in this condition (pH=4 and DO 5%) was estimated to be about 2 × 3 µ by light microscopy software (Figure 4). Again in this condition out of all the others, we found the biggest size for yeast cell for use as carriers in loading active materials. SEM images also confirmed the structural uniformity of yeast cells as carriers and the volume which is surrounded by the cell wall (Figure 5).

5. Conclusions

According to the results, the condition (pH=4 and DO 5%) is the best growth condition for *Saccharomyces cerevisiae* to have the best morphological properties. The importance of these findings is that this yeast can be regarded as an efficient and affordable carrier. It is suggested that in future studies, different active ingredients are encapsulated in the yeasts as carriers and their effectiveness and stability be studied.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

Both authors contributed to this project and article equally. Both authors read and approved the final manuscript.

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