Pre-evaporation of Ethanol as an Effective Method to Improve Single Cell Protein (SCP) Production from Date Palm Residue by Saccharomyces Cerevisiae

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Abstract. Unicellular proteins or Single cell proteins (SCP) are proteins that can be used for human foods and animal feed. Their source is microorganisms that grow unicellular, such as yeast, bacteria, mold, and algae. There are many carbon sources, which can be used in the production of a single cell protein (SCP). Many processes have been developed to produce SCP at a low cost. However, in all these processes, productivity was low. The current study aims at using a natural material (turnip extract) to increase the extraction of sugar from palm date residues required for yeast (Saccharomyces cerevisiae) fermentation. As well as the use of a vacuum pump to remove ethanol from fermentation broth at a certain time interval to increase the growth of the yeast and prevent its inhibition. From the study, the single-cell protein was increased from 30 gm to range between 60-90 gm, while the time of fermentation reduced from 24hr to 9 hr. The main fermentation experiments were performed using a 2 litter bioreactor at a moderate temperature 30 °C and pH value of 6. The use of residues, in addition to the production of single-cell proteins, allows to reduce of environmental pollution.

1. Introduction
Humankind is constantly trying to overcome the growing crises of steady population growth, declining quality of life, poverty, and hunger by developing technology that may provide new food resources. In light of the technological development, it is possible to make reliable estimates of sufficient resources for the population growth, which could reach 9.3 billion people by the year 2050, which will lead to a steady increase in food and water consumption. The scarcity of protein resources during these growing stages of growth is a source of serious concern to the world due to the limited availability of these resources [1], and the problem of global warming, and human health issues [2-3].

To overcome the problem of protein deficiency, the production of microbial protein, also known as the single-cell protein has a great advantage because it does not affect the soil and it is independent of the climate [4].

Many sources define Single-cell proteins SCPs as the dried cells of microorganisms such as fungi, algae and bacteria that are used as a protein supplement to human food or animal feed by using waste-based products, i.e. any inexpensive materials as carbon sources [5]. SCP is produced by using algae, yeast, fungi, and bacteria as a food supplement to replace conventional protein like soymeal and...
fishmeal [6-7]. Single-cell protein (SCP) is produced by using the fermentation process through the use of a specific strain of microorganisms that grow and multiply on certain media and substrates. The basic steps for fermentation process include availability of media with a carbon source, avoiding contamination of both fermenter and media, an examination of microorganisms, selection of the best technology that takes into account economic viability without any safety concerns [8-9]. The fermentation process for single-cell protein production includes according to [9].

Mass doubling time and average composition for microorganisms respectively were given by [10-11].

- A pure culture of the selected microorganism that is in the correct physiological state.
- Sterilization of the growth medium which is used for the organism.
- A production fermenter which is the equipment used for drawing the culture medium in a steady state.
- Cell separation.
- Collection of cell-free supernatant.
- Product purification.
- Effluent treatment

The choice of the yeast type for SCP production depends on the availability of the nutrient and the absence of inhibitors in the culture medium [12]. Also, yeast contains certain percentages of proteins and fats, in addition to smaller amounts of vitamins, minerals, and amino acids [13]. Yeasts can convert sugar in any form, whether sugar cane juice or molasses sugar, into ethanol. There is still a portion of the sugar that yeast uses for cellular metabolism to preserve and grow [14]. Many materials are used as a substrate or source of carbon that microorganisms use to produce SCP, such as wheat straw, orange peels, sugar cane, date waste, wood, or any other agricultural waste [15-16]. Some sources contain cellulose and hemicellulose, which can be used as a source, but they need chemical or physical processes to break down the bonds and convert them into simple sugars so that they do not cause a certain resistance to the action of microorganisms, that will cause the production of non-ethanol compounds [7].

Depending on the amount of sugar transformed, the nature of the compounds produced may differ according to the operating conditions of each industrial unit. Some research indicates that 5% of the sugar in the fermentation process converted into ethanol. An increase in the amount of yeast produced occurs as a result of the multiplication of the original cells during growth, and therefore every liter of ethanol produced corresponds to an increase in the mass of yeast of 50 grams, which can be removed without affecting production [17].

The role of yeasts in the production of fermented foods well established, Saccharomyces cerevisiae is the main model of yeast in studies and research. It also considered one of the microorganisms that do not contain chlorophyll and can resist chemical decomposition in addition to being quick to spread with water and stay in it for a reasonable period. Yeasts are not nutritionally demanding compared to other microorganisms such as lactic acid bacteria [18-19].

The use of new low-cost substrates as a raw material for the manufacture of single-cell protein SCP is very important in the industry, especially for countries that have high production of date, date residues. Saccharomyces cerevisiae was choose as the growing organism. Finding the optimum operating conditions by applying the simplex method was a target of the study to maximize product yield and protein content of the final product. Moreover, pre evaporation used to prevent the accumulation of ethanol to maximize production rate.

2. Materials and methods

2.1. Strains
Saccharomyces cerevisiae strains from the local market used in these experiments.
2.2. **Cultivation conditions**
Experiments performed under sterile conditions. 250 ml Erlenmeyer flasks containing 100 ml of sugar solution used. The incubation of yeast on malt extract performed for 5 hours at 30°C with mixing at 150 rpm. The sugar used was of about 15 brix.

2.3. **Date and turnip juice**
Date extract and offal are used as an alternative substrate for yeast (Saccharomyces cerevisiae) cultivation. The method of extracting sugar of dates was adapted from [20] with some modification. 250 gm of Zahdi date mixed with 750 ml of water and using a mixer for 1 hr at different temperatures, the mixture squeezed, reading its Brix grade. The weight of sugar extracted was 134.1 gm including 40% sucrose, 14% glucose, and 11% Fructose. While for turnip, 100 gm of turnip washed and dried then catted to small pieces and added 600 ml of water, put in an oven at 60°C, for 30 min. squeezed to take the only solution, which was 680 ml and Its Brix, was around 0.6-0.8. Date residues 100 gm of date residues were again added to 1000 ml of water and put in an oven at 60°C for 10 min, then rise temperature to 95°C for 1 hr. Sugar extracted here was about 13.2 g and its Brix was 11.

2.4. **Units and equipment**
Main units, equipment, and devices that used during this study included a bioreactor 2 l, Incubator, cooling-Shaking incubator, Shaking water bath, Oven, Spectrophotometer, Autoclave, Hot plate with a magnetic stirrer, Vortex, Micropipettes, and Sensitive balance.

2.5. **Cultivation of saccharomyces cerevisiae in batch bioreactor**
A fermenter with a capacity of 2 litters was used to grow the yeast under a pH of 6. The fermenter filled with a liquor (sugar solution) at 15 Brix and 30 ml of turnip extract solution added. 15 gm of yeast added to the fermenter after cultivation. After the beginning of the fermentation process and for periods 30, 60, 80, 100, and 120 minutes, ethanol withdrawn for one minute at 37°C under a vacuum pressure of 14 kPa. Operating conditions of temperature and stirring speed controlled during the experiments. Regulating the rate of airflow into the fermenter done through a distribution tube submerged inside the fermenter below the mixture. Air passed through a sterile 0.2-micrometer filter before entering the fermenter. Bubbles cause an increase in the mixing process in addition to the presence of the mixer. Two runs done, one without withdrawal, the other with withdrawal of ethanol.

2.6. **Sugar quantification**
On fermentation proceeds, sugar estimation via its Brix recorded; also, pH of the mixture recorded, to give another way to estimate sugar concentration. A different reading of pH at time intervals recorded. The amount of sucrose estimated by calculating the amount of glucose present after fermentation for each sample minus the amount of glucose before fermentation.

3. **Results and discussion**
3.1. **Production of yeast**
Figure 1 illustrates the duplication of yeast during fermentation. As we removed ethanol from fermentation media, it will cause yeast to keep growing so we have large yeast productivity. While if we do not remove ethanol, its accumulation in the fermentation broth causes a defect in yeast to grow, as higher the percentage of ethanol in the fermentation medium, the rate of production and growth of yeast decreases, and thus its ability to grow and multiply is inhibited. This is consistent with published research, where at 15 grams per litter, inhibition of the yeast begins [21-22]. In fact, at high concentrations of alcohol, the enzymes that cause metabolism begin to lose their potency, and then the yeast walls break down and lose the ability to grow or survive [23].
3.2. Production of ethanol and carbon dioxide

Figures 2, 3, and 4 show the productivity of ethanol and carbon dioxide, and the gradient of the sugar level in the fermentation medium. The alcoholic fermentation process is one of the most well-known fermentation processes, during which many transformations occur by the action of different enzymes, and the yeast begins to grow and metabolize to produce ethanol and carbon dioxide. When alcohol accumulates, the yeast is inactivated, the growth rate and the production of ethanol decrease, and thus the ability of cells to reproduce reduced. These results are in agreement with [24]. In addition, as a high level of sugar, exist, in case we do not withdraw ethanol, the yeast losses its ability to multiply and so, a high level of sugar remains.

**Figure 1.** Yeast productivity with and without ethanol withdraw

**Figure 2.** Ethanol production during fermentation.
3.3. PH variation

The decrease in the pH value as shown in Figure 5 attributed to the interference of carbonate and bicarbonate ions associated with water hardness as an effective factor to reduce the pH. When the fermentation process begins, a decrease in the pH value occurs due to a change in the mineral composition inside the solution. This occurs due to the degradation process of the agricultural medium and its components, and there is a deposition of phosphates and some amino acids, and this is consistent with what has been found from other researchers [25-27]. Figures 6-7 shows yeast production after the fermentation process is completed.

Figure 3. Carbon dioxide produced during fermentation.

Figure 4. Sugar content during fermentation.

Figure 5. PH variation during fermentation.
4. Conclusions
Agricultural waste, including date remnants, is one of the main resources for producing single-cell protein, as well as ethanol, which is an economical method that does not include high costs and reduces pollution. In this aspect, a lot of agricultural waste is usable. Date juice used as economically effective. SCP increased from 30 gm to 90 gm especially when ethanol withdrawn simultaneously. Furthermore, we need to design a filter medium effective in the passing of ethanol only during vacuum withdrawal; this could be the next important issue for the next studies.

Figure 6. Some apparatus used in the research.

Figure 7. Yeast produced after fermentation.
References

[1] Verstraete, W., Clauwaert, P., & Vlaeminck, S. E. (2016). Used water and nutrients: Recovery perspectives in a 'panta rhei' context. Bioresource technology, 215, pp. 199-208.

[2] Tursi, A. (2019). A review on biomass: importance, chemistry, classification, and conversion. Biofuel Research Journal, Vol. 6, No. 2, pp. 962-979.

[3] Watts, N., Amann, M., Arnell, N., Ayeb-Karlsson, S., Belesova, K., Berry, H., & Campbell-Lendrum, D. (2018). The 2018 report of the Lancet Countdown on health and climate change: shaping the health of nations for centuries to come. The Lancet, 392 (10163), pp. 2479-2514.

[4] Watts, N., Amann, M., Ayeb-Karlsson, S., Belesova, K., Bouley, T., Boyko, M., & Cox, P. M. (2018). The Lancet Countdown on health and climate change: from 25 years of inaction to a global transformation for public health. The Lancet, Vol. 391, No. 10120, pp. 581-630.

[5] Hülsen, T., Hsieh, K., Lu, Y., Tait, S., & Batstone, D. J. (2018). Simultaneous treatment and single cell protein production from agri-industrial wastewaters using purple phototrophic bacteria or microalgae—a comparison. Bioresource technology, pp. 254, 214-223.

[6] Yunus, F. U. N., Nadeem, M., & Rashid, F. (2015). Single-cell protein production through microbial conversion of lignocellulosic residue (wheat bran) for animal feed. Journal of the Institute of Brewing, Vol. 121, No. 4, pp. 553-557.

[7] Ahmed, P. M., Fernández, P. M., De Figueroa, L. I., & Pajot, H. F. (2019). Exploitation alternatives of olive mill wastewater: production of value-added compounds useful for industry and agriculture. Biofuel Research Journal, Vol. 6, No. 2, pp. 980-994.

[8] Bajpai, P. (2017). Single cell protein production from lignocellulosic biomass. Singapore: Springer.

[9] Chandrani-Wijeyeratne, S., & Tayathilake, A. N. (2000). Characteristics of two yeast strain (Candida tropicalis) isolated from Caryotaurens (Khitul) today for single cell protein production. J. Natl. Sci. Found. Sri Lanka, Vol. 28, pp. 79-86.

[10] Nasser, A. T., Rasoul-Amini, S., Morowvat, M. H., & Ghasemi, Y. (2011). Single cell protein: production and process. American Journal of food technology, Vol. 6, No. 2, pp. 103-116.

[11] Bajpai, P. (2017). Single cell protein production from lignocellulosic biomass. Singapore: Springer.

[12] Miller, B. M., & Litsky, W. W. (1976). Single Cell Protein in Industrial Microbiology.

[13] Bekatorou, A., Psarianos, C., & Koutinas, A. A. (2006). Production of food grade yeasts. Food technology and biotechnology, Vol. 44, No. 3, pp. 407-415.

[14] Pires, J. F., Ferreira, G. M., Reis, K. C., Schwan, R. F., & Silva, C. F. (2016). Mixed yeasts inocula for simultaneous production of SCP and treatment of vinasse to reduce soil and fresh water pollution. Journal of environmental management, Vol. 182, pp. 455-463.

[15] De Menezes, T. J. B. (1980). Etanol, o combustível do Brasil. Editora Agronômica Ceres.

[16] Suman, G., Nupur, M., Anuradha, S., & Pradeep, B. (2015). Single cell protein production: a review. Int. J. Curr. Microbiol. App. Sci, Vol. 4, No. 9, pp. 251-262.

[17] Lapeña, D., Kosa, G., Hansen, L. D., Mydland, L. T., Passoth, V., Horn, S. J., & Eijsink, V. G. (2020). Production and characterization of yeasts grown on media composed of spruce-derived sugars and protein hydrolysates from chicken by-products. Microbial cell factories, Vol. 19, No. 1, pp. 1-19.

[18] Andrietta, MG, Steckelberg, C., Kitaka, PR, Goldebeck, R., & Andrietta, SR (2017). Yeast from ethanol production – source of scp (single cell protein). WJRR, Vol. 4. We are a family owned and operated business, pp. 87-89.

[19] Copetti, M. V. (2019). Yeasts and molds in fermented food production: an ancient bioprocess. Current opinion in food science, pp. 25, 57-61.

[20] Gallone, B., Steensels, J., Prahl, T., Soriaga, L., Saels, V., Herrera-Malaver, B., & Teiling, C.
(2016). Domestication and divergence of Saccharomyces cerevisiae beer yeasts. Cell, Vol. 166, No. 6, pp. 1397-1410.

[21] Nancib, N., Ghoul, M., Larous, L., Nancib, A., Adimi, L. Z., Remmal, M., & Boudrant, J. (1999). Use of date products in production of the thermophilic dairy starter strain Streptococcus thermophilus. Bioresource Technology, Vol. 67, No. 3, pp. 291-295.

[22] Luong, J. H. T. (1985). Kinetics of ethanol inhibition in alcohol fermentation. Biotechnology and bioengineering, Vol. 27, No. 3, pp. 280-285.

[23] Klinke, H. (2003). B.; Olsson, L.; Thomsen, AB; Ahring, BK Potential inhibitors from wet oxidation of wheat straw and their effect on ethanol production of Saccharomyces cerevisiae: Wet oxidation and fermentation by yeast. Biotechnology and Bioengineering, Vol. 81, pp. 738-747.

[24] Malakar, S., Paul, S. K., & Pou, K. J. (2020). Biotechnological Interventions in Beverage Production. In Biotechnological Progress and Beverage Consumption, Academic Press, pp. 1-37.

[25] Ciani, M., Comitini, F., & Mannazzu, I. (2013). Fermentation.

[26] Pou, K. J., Paul, S. K., & Malakar, S. (2019). Industrial Processing of CTC Black Tea. In Caffeinated and Cocoa Based Beverages, Woodhead Publishing, pp. 131-162.

[27] Muataz H. Ismael, Salah N. Farhan, Yaser I. Jasem, Walaa Abid Mahmood. (2020). International Journal on Emerging Technologies, Vol. 11, No. 2, pp. 845-848.