Journal of Harbin Engineering University
ISSN: 1006-7043

Vol 44 No. 9
September 2023

Production and Optimization of Astaxanthin by *Phaffia rhodozyma* under Submerged Fermentation

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Objective: One of the main processes for creating astaxanthin, an essential pigment with industrial and pharmacological applications, is *Phaffia rhodozyma* fermentation. The effects of nitrogen supply and carbon source on *Phaffia rhodozyma* culture and astaxanthin production were examined using single factor and mixture design experiments in an effort to increase astaxanthin productivity. The design of the fermentation medium is a crucial step in the creation of any metabolite. Plackett-Burman Using design in the first phase was an effective way to determine how production and medium components will interact. Significantly, the second step's usage of Box-Behnken's Design of Response Surface Methodology was helpful in figuring out the ideal concentration of the elements that significantly affect the formation of astaxanthin and biomass. The optimized final composition of the submerged fermentation medium used by MTCC 7536 to produce biomass and astaxanthin. This indicates that the projected value is 65 and 68% valid. Since the medium is chemically specified, it might not affect further processing. Due to its simpler processing and practical features like experimental design, 3D contours, 3D surface graphs, Point prediction, etc., the Design Expert 7.0 software utilized for the optimization study was determined to be a very effective tool for optimization.
Keywords: Astaxanthin, Nitrogen supply, Carbon source, *Phaffia rhodozyma*, Optimization

Introduction

The world's strongest natural antioxidant, astaxanthin The orange-red carotenoid astaxanthin (C40H52O4, molecular weight: 596.841 g/mol) is a member of the xanthophyll family of pigments [1]. The unusual carotenoid astaxanthin (3,3′-dihydroxy-,-carotene-4,4′-dione) is found in abundance in nature. It is a wonderful Xanthophyll family member. One of the main pigments in the carotenoid family, it is frequently employed to give some birds, crustaceans, and salmons their color [2].

Strong antioxidant astaxanthin works to scavenge singlet oxygen and prevent the production of free radicals, protecting the phospholipid cell membrane and other lipid components. The prevention of cancer, inflammation, and diabetes is one area where astaxanthin has significant promise and promising uses in nutrition and human health [3]. *Phaffia rhodozyma*, commonly known as Xanthophyllomyces dendrorhous, is the hetero basidiomycetous yeast that produces the majority of the primary astaxanthin during yeast fermentation. Future significant development in its industrial market share will be driven by extensive uses in medicines, human nutritional supplements, cosmetics, and feed additives [4]. Compared to lutein, lycopene, and -tocopherols, astaxanthin is a more potent antioxidant [5].

Media and Chemicals

Components for yeast malt (YM) medium were bought from Merck and Central Drug House in Mumbai. Analytical Grade chemicals were employed throughout the investigation, and Hi Media, Qualigens, Merck, and other reputable companies bought them from a number of well-known producers.

Phaffia rhodozyma MTCC 7536 strain, medium, and cultivation were acquired from MTCC, IMTECH, Chandigarh, India. It was subcultured for additional investigation and kept on the slants of yeast-malt agar (YMA) at 4°C. *P. Rhodozyma* MTCC 7536 seed culture spore suspension was made from yeast that was actively growing in YM broth (YMB). A 250 mL Erlenmeyer flask containing 50 mL of sterilized medium (10 g dextrose, 5.0 g peptone, 3.0 g malt extract, and 3.0 g yeast extract in 1000 mL distilled water; adjusted to pH 5.8) was inoculated with 5% *P. rhodozyma* spore suspension and incubated for 6 days at 200 rpm with a modification.

Yeast Seed Culture Preparation
Erlenmeyer flask (250 ml) containing 50 ml sterilized YM medium (10 g Dextrose, 5g Peptone, 3 g Malt extract, 3 g Yeast extract in 1000 ml Distilled water; adjusted to pH 5.8) were inoculated with spore suspension of *P.rhodozyma* and incubated at 22°C for 6 days in an orbital shaker incubator at 200 rpm [6].

Submerged Fermentation
In a 250 ml Erlenmeyer flask [12] with 50 ml of production medium (Malt Extract, Yeast Extract, Peptone, Beef Extract, Ammonium Sulphate, Casein Hydrolysate, Dextrose, Maltose, Sucrose, pH-5.8), submerged fermentation was performed.5% v/v of the seed culture was added to the sterilized production medium before being incubated at 22°C for 6 days at 200 rpm.
Using enzymes to extract astaxanthin
With some modifications, astaxanthin was extracted using the technique outlined by Michelon et al. [7]. 10 mL of the culture was pelleted down for 10 min. at 3000 rpm after fermentation. Following centrifugation, the pellet was treated with -glucanase (10 mg/mL) in 0.1 M sodium phosphate buffer (pH 7.0), with the supernatant being discarded and the pellet being shaken at 100 rpm for 8 hours at 35°C in the dark. It was centrifuged after being probe sonicated for 5 min. A UV-vis spectrophotometer operating at 450 nm was used to determine the amount of astaxanthin in the collected supernatant [8].

Astaxanthin and Biomass Estimation
Sedico Ltd.'s Double beam UV-Vis SpectroScan 80 DV (Sedico Ltd.) was used to measure the concentration and biomass of astaxanthin in the culture medium at wavelengths of 450 nm and 610 nm, respectively. Nine medium elements were chosen for the study: malt extract, yeast extract, peptone, beef extract, ammonium sulphate, casein hydrolysate, dextrose, maltose, and sucrose. The relative relevance of various nutrients for astaxanthin synthesis in submerged culture was assessed using the Plackett-Burman experimental design for eleven variables, nine nutritional components (independent variables), and two dummy factors. Two distinct concentrations (+) and a low concentration (-) were evaluated for each nutritional variable.

Response Surface Approach
Plackett-Burman Design screening resulted in the selection of three medium components for the study: peptone, maltose, and sucrose. Using the DESIGN EXPERT 7.0 program, an experimental design of 17 runs containing central points was created in accordance with Box-Behnken's response surface design for a set of three parameters. To achieve this, first make the YM medium and add 2 g of succinic acid per 100 ml, or 2% succinic acid. Box Behnken's Method is the technique employed in Response Surface Methodology. After the production medium has been prepared, adjust the pH to 5.8, inoculate the medium with 5% v/v (2.5 ml) seed culture, and incubate the mixture for 6 days at 22°C with an orbital shaker incubator running at 200 rpm.

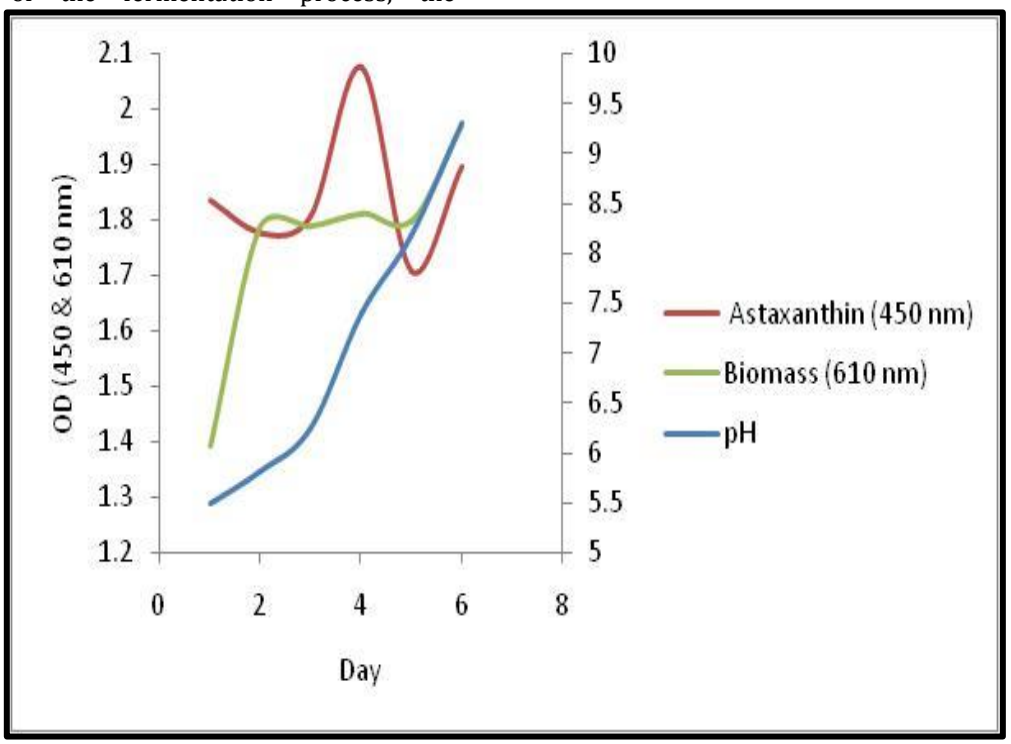
Process Kinetics During Fermentation
Prepare the 12 flasks of the optimum media for the kinetic investigation. For 1000 ml in the first six flasks (Peptone 5 g/l, Maltose 5 g/l, and Sucrose 5 g/l). Add the components of the YM medium to the second flask (6, peptide 15 g/l, maltose 5 g/l, and sucrose 15 g/l) for a total volume of 1000 ml.maintained the 12 flasks on an orbital shaker incubator at 22 °C and 200 rpm for 6 days. Spectrophotometer measurements of astaxanthin and biomass are made daily (days 1 through day 6) at 450 and 610 nm, respectively, and pH is also taken daily.
P. rhodozyma is being grown at a pilot scale in a 5 L in-situ lab fermentor.
Using *P. rhodozyma* UV mutant culture in a 5L container, the maximal yield of biomass in a pilot-scale fermentor was determined. The mechanically stirred tank fermentor (Scigenics, India Pvt. Ltd.), with a total volume of 5L and three impellers, each with six last-bladed disc turbines on the vertical shaft, was used for laboratory-scale fermentation research. The temperature of the vessels was managed by water pushed via jackets. 250 Erlenmeyer flasks were used to create the inoculum by transferring a loop of cells from 6-day-old slant cultures. 300 cc of the seed culture is injected into a three-litre batch of production media. The flask was then held at 22°C for 6 days on a rotary shaker running at 200 rpm. A working volume of 3L of this production medium was inoculated in the lab fermentor.

Separation of the Yeast Biomass and Astaxanthin Extraction
For the separation of yeast biomass, fermented broth was collected and centrifuged at 300 rpm. Separated yeast cells were processed using a variety of techniques, including freeze-thaw at -20°C for 12 hours, repeated twice (2 cycles), steam treatment at 121°C for 15 minutes, microwave treatment at 650 watts for 2 minutes, and enzyme lyticase treatment for wet biomass. Wet mass was used to treat the lyticase enzyme for 8 hours at a concentration of 10 mg/ml in phosphate buffer at pH 7.0 and 35 °C. 2 millilitres of the enzyme solution per gram of pellet weight.

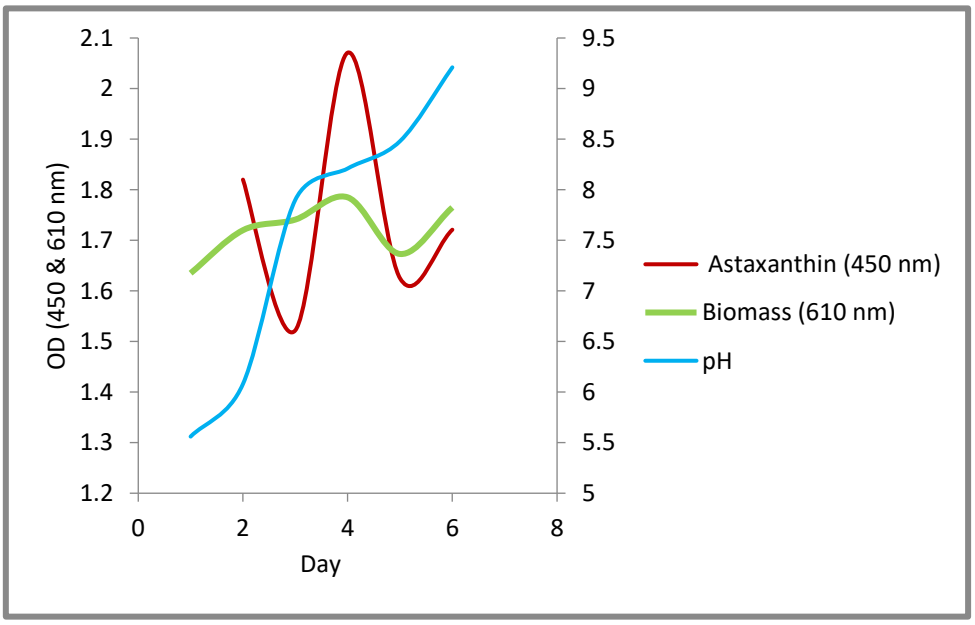
Findings and Discussion

Process Kinetics During Fermentation

While the concentration of biomass was observed to increase significantly from day 1 to day 2 of the fermentation process, the concentration of astaxanthin originally remained constant. Later, the biomass concentration remained steady till day 4 while astaxanthin concentration showed an increase (Fig. 1).



As the fermentation process progressed, it was discovered that the concentration of biomass increased marginally from day 1 to day 3 while the concentration of astaxanthin increased dramatically from day 3 to day 4. Later, the biomass content was seen to have decreased (Fig. 2).



Production of Astaxanthin in a 5L in-situ Laboratory Fermentor

Two batches of rum were made in a lab fermentor under the optimal circumstances, which produced 288 g of wet yeast pellets from 3L of medium

Sr.No	Operation Conditions	
1	pH profile was maintained 5.8	2 N HCL 2 N NaOH
2	Agitation speed	300 rpm
3	Dissolved oxygen	40%
4	Inoculum volume	10 %
5	Elicitation: Succinic acid	2 %
6	Antibiotics ml	Ampicillin (1 mg/ml) 15 Fluconazole (1mg/ml) 15 ml
7	Aeration ratio	10 LPM
8	Time	6 days
9	Temperature	22˚C
10	Media	Optimized Media

Figure : 3

a. Submerged fermentation of P. rhodozyma in 5 L-in-situ lab fermentor.
b. Astaxanthin pellet after 6 days of fermentation in optimized media.
c. Lyophilized powder for extraction. separation of the yeast biomass and astaxanthin extraction
Separation of Yeast biomass and Extraction of astaxanthin
Table 1.: Biomass and astaxanthin content analysed at every step of Extraction

Sr. No Treatment Biomass (OD 610 nm) Astaxanthin (OD 450 nm)

1 Freeze thaw 2.355 0.584
2 Microwave assisted 2.600 2.416
3 Autoclave 2.838 1.893
4 Enzyme (Lyticase)3.205 1.51

Conclusion

The design of the fermentation medium is a crucial step in the creation of any metabolite. Plackett-Burman Using design in the first phase was an effective way to determine how production and medium components will interact. Significantly, the second step's usage of Box-Behnken's Design of Response Surface Methodology was helpful in figuring out the ideal concentration of the ingredients that significantly affect the production of astaxanthin and biomass. the optimized composition of the submerged fermentation medium used to produce biomass and astaxanthin. This material projects astaxanthin to be 1.99 and biomass to be 1.253. In the afore mentioned optimized fermentation media, practically 3 was attained. This indicates that the projected value is 65 and 68% valid. Since the medium is chemically specified, it might not affect further processing. Due to its simpler processing and practical features like experimental design, 3D contours, 3D surface graphs, Point prediction, etc., the Design Expert 7.0 software utilized for the optimization study was determined to be a very effective tool for optimization.

References

1. Elwan, H.A.M., Elnesr, S.S., Abdallah, Y., Hamdy, A. and El-Bogdady, A.H., 2019. Red yeast (Phaffia rhodozyma) as a source of astaxanthin and its impacts on productive performance and physiological responses of poultry. World's Poultry Science Journal, 75(2), pp.273-284.
2. Wang, C., Armstrong, D. W., & Chang, C. D. 2008. Rapid baseline separation of enantiomers and a mesoform of all-trans-astaxanthin, 13-cis-astaxanthin, adonirubin, and adonixanthin in standards and commercial supplements. Journal of Chromatography A, 1194(2), 172-177.
3. Mularczyk, M., Michalak, I. and Marycz, K., 2020. Astaxanthin and other nutrients from Haematococcus pluvialis—Multifunctional applications. Marine drugs, 18(9), p.459.
4. Keerthi, R.S., Dhabliya, D., Elangovan, P., Borodin, K., Parmar, J., Patel, S.K. Tunable high-gain and multiband microstrip antenna based on liquid/copper split-ring resonator superstrates for C/X band communication (2021) Physica B: Condensed Matter, 618, art. no. 413203, .
5. Verdoes, J. C., Misawa, N., & van Ooyen, A. J. (1999). Cloning and characterization of the astaxanthin biosynthetic gene encoding phytoene desaturase of Xanthophyllomyces dendrorhous. Biotechnology and bioengineering, 63(6), 750-755.
6. Vadivu, N.S., Gupta, G., Naveed, Q.N., Rasheed, T., Singh, S.K., Dhabliya, D. Correlation-Based Mutual Information Model for Analysis of Lung Cancer CT Image (2022) BioMed research international, 2022, p. 6451770.
7. Guerin, M., Huntley, M. E., & Olaizola, M. (2003). Haematococcus astaxanthin: applications for human health and nutrition. TRENDS in Biotechnology, 21(5), 210-216.
8. Hussein, G., Goto, H., Oda, S., Sankawa, U., Matsumoto, K., & Watanabe, H. (2006). Antihypertensive potential and mechanism of action of astaxanthin: III. Antioxidant and histopathological effects in spontaneously hypertensive rats. Biological and Pharmaceutical Bulletin, 29(4), 684-688.
9. Bjerkeng, B., Storebakken, T., & Liaaen-Jensen, S. (1990). Response to carotenoids by rainbow trout in the sea: resorption and metabolism of dietary astaxanthin and canthaxanthin. Aquaculture, 91(1), 153-162.
10. Shimidzu, N., Goto, M., & Miki, W. (1996). Carotenoids as singlet oxygen quenchers in marine organisms. Fisheries science, 62(1), 134-137.
11. Kothandaraman, D., Praveena, N., Varadarajkumar, K., Madhav Rao, B., Dhabliya, D., Satla, S., Abera, W. Intelligent Forecasting of Air Quality and Pollution Prediction Using Machine Learning (2022) Adsorption Science and Technology, 2022, art. no. 5086622,.
12. Johnson, E. A., & Schroeder, W. A. (1995). Microbial carotenoids. In Downstream processing biosurfactants carotenoids (pp. 119-178). Springer Berlin Heidelberg.
13. Michelon, M., de Borba, T. D. M., da Silva Rafael, R., Burkert, C. A. V., & de Medeiros Burkert, J. F. (2012). Extraction of carotenoids from Phaffia rhodozyma: A comparison between different techniques of cell disruption.Food Science and Biotechnology, 21(1), 1-8.
14. Liu, X., Xie, J., Zhou, L., Zhang, J., Chen, Z., Xiao, J., Cao, Y. and Xiao, H., 2022. Recent advances in health benefits and bioavailability of dietary astaxanthin and its isomers. Food Chemistry, p.134605.
15. Bjørklund, G., Gasmi, A., Lenchyk, L., Shanaida, M., Zafar, S., Mujawdiya, P.K., Lysiuk, R., Antonyak, H., Noor, S., Akram, M. and Smetanina, K., 2022. The Role of Astaxanthin as a Nutraceutical in Health and Age-Related Conditions. Molecules, 27(21), p.7167.
16. Venu, S., Kotti, J., Pankajam, A., Dhabliya, D., Rao, G.N., Bansal, R., Gupta, A., Sammy, F. Secure Big Data Processing in Multihoming Networks with AI-Enabled IoT (2022) Wireless Communications and Mobile Computing, 2022, art. no. 3893875, .
17. Mehraj, H., Jayadevappa, D., Haleem, S.L.A., Parveen, R., Madduri, A., Ayyagari, M.R., Dhabliya, D. Protection motivation theory using multi-factor authentication for providing security over social networking sites (2021) Pattern Recognition Letters, 152, pp. 218-224.