META-ANALYSIS FOR SINGLE CELL OIL BY *Rhodotorula glutinis*: SUBSTRATES FROM INDUSTRIAL WASTE

Luiz C. de Oliveira Junior[1], Jaciane L. Ienczak[1], Eloísa C. da Silva[2] & Acácio F. Zielinski[1]

[1]Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, Brazil
[2]Veterinary Medicine Course, Federal Institute of Santa Catarina, Concórdia, Brazil
** Corresponding author's email address: engluizcarlosjunior@gmail.com*

ABSTRACT

Oleaginous yeasts produce and store lipids, known as Single Cell Oil (SCO). Within this group, we highlight the productivity of *Rhodotorula glutinis*, a yeast capable of adapting to various culture media and cultivation conditions. This SCO can address a significant problem we are facing: the environmental impact of fuel production using finite natural resources. Therefore, it is essential to develop and optimize the processes for obtaining SCO, making it increasingly viable both financially and ecologically. In this context, this biblioghaphy revision presents comparisons between cultives in different substrate sources, demonstrating that it is possible to reuse waste, thus making the process viable within the concept of upcycling, reducing costs, and becoming a less environmentally aggressive process.

Keywords: Yeast; SCO; *Rhodotorula glutinis*; Waste.

1 INTRODUCTION

Oleaginous yeasts produce intracellular lipids, which are referred to as Single Cell Oil (SCO) or microbial oil.[1] Currently, conventional fuels are causing significant environmental problems due to the use of finite natural resources for their production. Consequently, there is an increasing search for alternatives to replace products previously derived from these resources.[1, 2, 3] SCO can be used as a substitute.[4] Additionally, high-value SCO, a lipid intended for noble purposes, can be used in the food, pharmaceutical, and cosmetics industries, due to its composition, which includes nutrients such as vitamins, polyunsaturated fatty acids and carotenoids.[5, 6]

Numerous experiments have been conducted to determine, the best media and cultivation conditions. Along with this, it is crucial to identify the best yeasts for cultives process. An important factor for the viability of using yeasts in SCO production is their ability to grow over a wide pH range, reducing the impact of the process and facilitating cultivation conditions.[7, 8] One of the yeasts if have a good potential for this process is *Rhodotorula glutinis*.

For a reliable and visual method of comparison for a bests conditions in cultives, a good option is meta-analysis, a tool that seeks to integrate the best results obtained under different conditions while addressing the same scientific question.[9] *Rhodotorula glutinis* produces amounts of high-value SCO, which is primarily composed of high levels of fatty acid content of triacylglycerols such as of oleic, palmitic, and linoleic acids,[6] giving it potential for industrial-scale use.[10] This addresses another challenge faced by industries, which is the search for low-cost alternatives to manage waste and the high costs associated with these processes. Depending on the composition of the waste generated by the food and cosmetics industries, this wastes can become an excellent substrate source for oleaginous yeasts, being essential for cultivation as a carbon source.[11, 12, 13, 14]

Along with the study of the feasibility of synthetic or waste substrates, it is necessary to pay attention when planning the cultivation, as the to the production of SCO during cultivation occurs only at high C/N ratios, causing nitrogen to become limited in the medium, which triggers lipid accumulation.[3, 6] It is also important to highlight the high cost of carbon sources from non-waste origins, which can constitute up to 72% of the total process cost. Therefore, the use of industrial waste, besides being significant considering the environmental impact of waste treatment and subsequent disposal, offers an economic advantage for the cost and feasibility of SCO production. Utilizing waste in a system with a biorefinery concept transforms it into a circular economy process.[15]

This review aimed to elucidate and analyze studies that utilized *Rhodotorula glutinis* in cultivation using waste as a substrate source, aiming to fill a gap in the literature.

2 MATERIAL & METHODS

A graph was evaluated through meta-analysis using the software "Excel". For the construction of this graph, the Scopus and Web of Science databases were used, yielding 107 articles: 56 from Scopus and 51 from Web of Science. The keywords used were: "Rhodotorula" AND "glutinis" AND "Single" AND "Cell" AND "Oil" AND "Waste". Eight articles were selected for the development of the meta-analysis graph, considering the substrate source, batch type, and C/N ratio. An additional 11 articles were used to provide theoretical support for this review.

3 RESULTS & DISCUSSION

Rhodotorula glutinis can grow in a variety of cultures media, using several diferente carbon source (Table1).

Table 1 Substrate concentration, nitrogen and C/N ratio in *Rhodotorula glutinis* cultivation.

Substrate source	Substrate concentration (g/L)	Nitrogen concentration (g/L)	C/N ratio (w/w)	Reference
Supplemented Date Syrup	70	1.00	70	3
Residual water from glycerol-supplemented potato	30	5.00	6	7
Sugarcane molasses	80	1.00	80	8
Glucose AR	60	1.00	60	8
Glycerol AR	31	0.30	10	15
Residual glycerol from biodiesel	31	0.30	103	15
Residual glycerol from biodiesel	40	0.35	113	17
Sugarcane molasses	40	0.35	113	17
AWBH - Wheat Bran Hydrolysate	31	0.17	188	18
OMW - Fresh Olive Mill Waste (50%)	24	0.17	145	18
Residual glycerol from biodiesel	30	0.17	132	18

A comparison did with two different types of commercial substrates: PA glucose and commercial glycerol. The residues used most commonly by researchers were sugarcane molasses and primarily crude glycerol, a byproduct resulting from biodiesel production via the transesterification route, which can constitute up to 10% of the total volume utilized in a process of biodiesel obtention.[19]

There was a variation in the amount of substrate consumed by the yeasts. This significantly affects the final cell concentration of the medium, where the highest concentration was achieved with the largest amount of substrate consumed. However, what determines the final concentration of lipids is not only the nitrogen limitation, but also the availability of abundant substrate in the medium. For lipid accumulation to occur, the nitrogen concentration must become limited, ceasing cell growth and triggering lipid accumulation within the cell. This phenomenon results from a mechanism in which the cell shifts from primary metabolism to secondary metabolism. When this occurs, SCO and carotenoids are produced simultaneously, enhancing the value of the cultivated product.[3, 6]

Table 1 shows the varying carbon-to-nitrogen ratios. Even with different initial carbon concentrations, experiments with similar C/N ratios yielded comparable lipid percentages at the end of the cultivation.

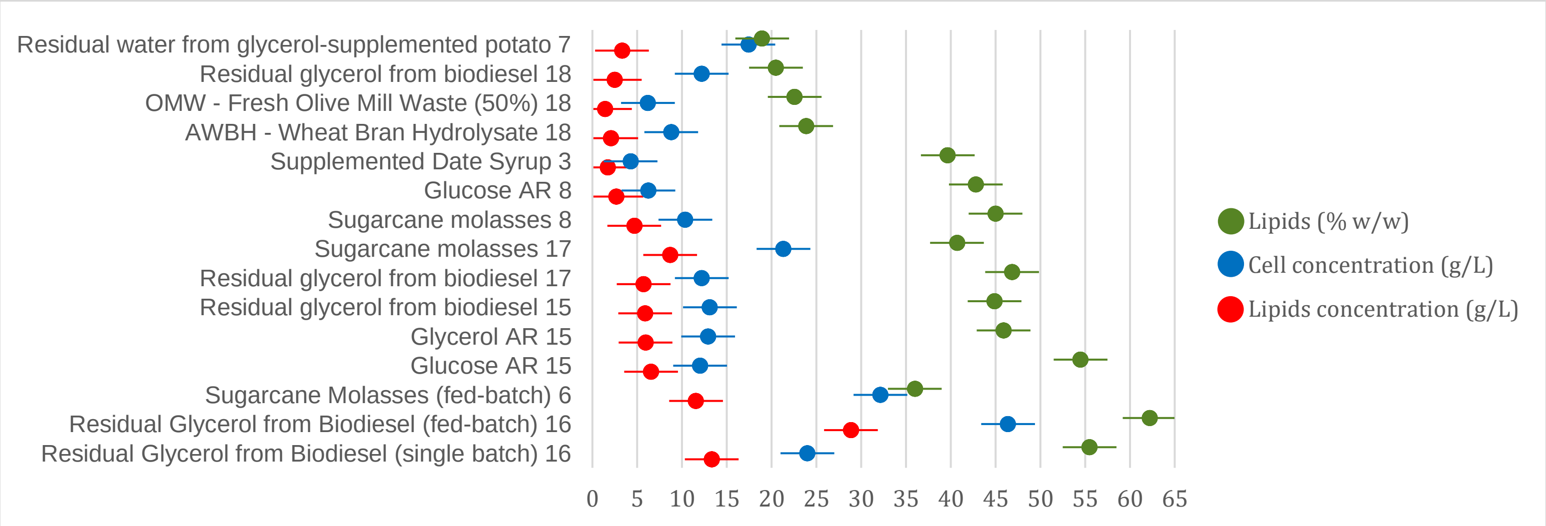

Figure 1 Result of meta-analysis, comparing cell concentration, lipid concentration and percentage of lipids in *Rhodotorula glutinis*.

Regarding the carbon source and the feeding method of the medium, similar growth was observed in the majority of studies, according to the amount of substrate available and the cultivation time. As for the method of feeding them medium, the best cell and lipid concentrations were obtained from fed-batch experiments, where a substrate pulse was added at the moment of nitrogen limitation, leaving the medium nitrogen-limited and with an excess of carbon for SCO production.

With similar final lipid concentrations (Figure 1), regardless of whether the substrate was sintetic or waste, the potential for developing industrial-scale processes using waste as a carbon source highlight . There was no significant discrepancy in the results in cells and lipid concentrations obtained from residual sources that would cause a substantial loss in cultives process productivity.

4 CONCLUSION

We conclude that *R. glutinis* is an excellent option for the production of SCO in alternative sources of substrate. Not only does it exhibit excellent substrate conversion to SCO, but it also adapts to various carbon sources, opening doors for the development of research, mainly in Brazil, where the agribusiness corresponds to 22.7% of the gross domestic product, and several wastes are obtained from this activity. This aligns with environmental, social and governance directions, focusing on processes involving microorganisms while striving to minimize environmental impact as much as possible.

REFERENCES

1 DIAMANTOPOULOU, P., SARRIS, D., TCHAKOUTEU, S.S., XENOPOULOS, E., PAPANIKOLAOU, S. GROWTH. 2023. Microorganisms, 11, 1863. https://doi.org/10.3390/microorganisms11071863.

2 ABELN, F., CHUCK, CJ. 2021. *Microb. Cell Fato.* 20, 221. https://doi.org/10.1186/s12934-021-01712-1

3 GHASEMI, A., MOOSAVI-NASAB, M. 2020. Biotechnol. Rep., 27, 1-8. http://dx.doi.org/10.1016/j.btre.2020.e00480.

4 GALLEGO-GARCÍA, M., SUSMOZAS, A., NEGRO, M. J., MORENO, A. D. 2023. *Microb. Cell Fato.* 22, 246. https://doi.org/10.1186/s12934-023-02254-4

5 LI, Z., LI, C., CHENG, P., YU, G. 2022. Heliyon, 8, 11, 1-18. http://dx.doi.org/10.1016/j.heliyon.2022.e11505.

6 VIEIRA, J. P. F., IENCZAK, J. L., ROSSELL, C. E. V., PRADELLA, J. G. C., FRANCO, T. T. 2014. *Biotechnol. Lett.* 36, 2433–2442. https://doi.org/10.1007/s10529-014-1624-0.

7 KOT, A. M., BŁAŜEJAK, S., KURCZ, A., BRYŚ, J., GIENTKA, I., BZDUCHA-WRÓBEL, A., MALISZEWSKA, M., RECZEK, L. 2017. Electron. J. Biotechnol., 27, 25-31. http://dx.doi.org/10.1016/j.ejbt.2017.01.007.

8 LAKSHMIDEVI, R., RAMAKRISHNAN, B., RATHA, S. K., BHASKAR, S., CHINNASAMY, S. 2021. Environ. Technol Inno. 21, 101281. http://dx.doi.org/10.1016/j.eti.2020.101281.

9 SIEFERT, A., VIOLLE, C., CHALMANDRIER, L., ALBERT, C. H., TAUDIERE, A., FAJARDO, A., AARSSEN, L. W., BARALOTO, C., CARLUCCI, M. B., CIANCIARUSO, M. V. 2015. Ecol. Lett., 18, 12, 1406-1419. Wiley. http://dx.doi.org/10.1111/ele.12508.

10 MAZA, D. D., BARROS, J. M., Guillamón, J. M., Aybar, M. J., Viñarta, S. C. 2024. Fermentation. 10, 178. https://doi.org/10.3390/fermentation10040178.

11 CHATURVEDI, S., BHATTACHARYA, A., NAIN, L., PRASANNA, R., KHARE, S. K. 2019. Biom. And Bioe., 127, 105294. http://dx.doi.org/10.1016/j.biombioe.2019.105294.

12 GHAZANI, S. M., MARANGONI, A. G. 2022. Trends. Food. Sci. Technol., 119, 593-607. http://dx.doi.org/10.1016/j.tifs.2021.10.014.

13 GÓMEZ, M. J. R., MAESTRO-GAITÁN, I., MAGRO, P. C., SOBRADO, V. C., BLÁZQUEZ, M. R., PRIETO, J. M. 2023. Int. Food Res. 164, 112160. http://dx.doi.org/10.1016/j.foodres.2022.112160.

14 UğUR, Ş., ZIENIUK, B., FABISZEWSKA, A. 2024. Appl. Sci. 14, 10, 4232. MDPI AG. http://dx.doi.org/10.3390/app14104232.

15 ANGELICOLA, M. V., FERNÁNDEZ, P. M., AYBAR, M. J., VAN NIEUWENHOVE, C. P., FIGUEROA, L. I. C., VIÑARTA, S. C. 2023. Biocatal. Agric. Biotechnol. 47, 102544. https://dx.doi.org/10.1016/j.bcab.2022.102544.

16 YEN, H., LIU, Y. X., CHANG, J. 2015. J. Taiwan Inst. Chem. Eng. 49, 67-71. http://dx.doi.org/10.1016/j.jtice.2014.11.019.

17 SINELI, P. E., MAZA, D. D., AYBAR, M. J., FIGUEROA, L. I. C., VIÑARTA, S. C. 2022. Energy Convers. Manag. X, 16, 100331. http://dx.doi.org/10.1016/j.ecmx.2022.100331.

18 GUERFALI, M., AYADI, I., AYADI, W., MAGRO, S., ELHADEF, K., ZAGHDEN, O., JLAIEL, L., SAHLI, E., BELGHITH, H., GARGOURI, A. 2024. Conv. Biomassa Bioref. 14, 1023710250. https://doi.org/10.1007/s13399-022-03028-5.

19 SANTANA, K. O. **Reaproveitamento do glicerol bruto de usinas de biodiesel em processos fermentativos de geração de H2.** 2017. 123 f. Dissertação (Mestrado) - Curso de Biotecnologia, Universidade Estadual Paulista, Araraquara, 2017.

ACKNOWLEDGEMENTS

They also acknowledge Foundation Coordination for the Improvement of Higher Education Personnel - CAPES for the support provided through process 88887.823128/2023-00.