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Dichloromethane as an alternative to chloroform in the extraction of single cell oil from Rhodotorula toruloides

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Abstract
Dichloromethane was evaluated as an alternative to chloroform in lipid extraction. The results indicate that dichloromethane could be used without compromise on extraction efficiency.

Key words:
Rhodotorula toruloides, single cell oil, lipid extraction

Introduction

In the face of the new environmental policies regarding atmospheric pollution, many studies concerning new green fuels are in progress. New alternative energy sources such as biodiesel obtained from single cell oil (SCO), however, require lipid extraction steps in their production processes.

The objective of this study was to assess the application of dichloromethane (CH_2Cl_2) as an alternative to chloroform ($CHCl_3$) in the extraction of single cell oil from the yeast *Rhodotorula toruloides*. According to Cequier-Sánchez[1] et al. (2016), dichloromethane is nearly 15% less expensive, its boiling point is 21.6 ºC degrees lower, and its toxicity threshold to human health is approximately 20 times higher compared to chloroform, thus qualifying as a potentially more ecological alternative.

Results and Discussion

Samples of 0.3 g of dried cells, were prepared as described by Bonturi[2] (2016) and soaked in 2:1 chloroform-methanol (C:M) and 2:1 dichloromethane-methanol (D:M) solutions. Cell *debris* was then filtered out of the suspensions, and the filtrate was evaporated. The remaining SCO was then weighed.

A 2^3 factorial experiment in triplicates (Chart 1) was designed in Minitab 7, with the mass fraction of extracted lipids as the response variable.

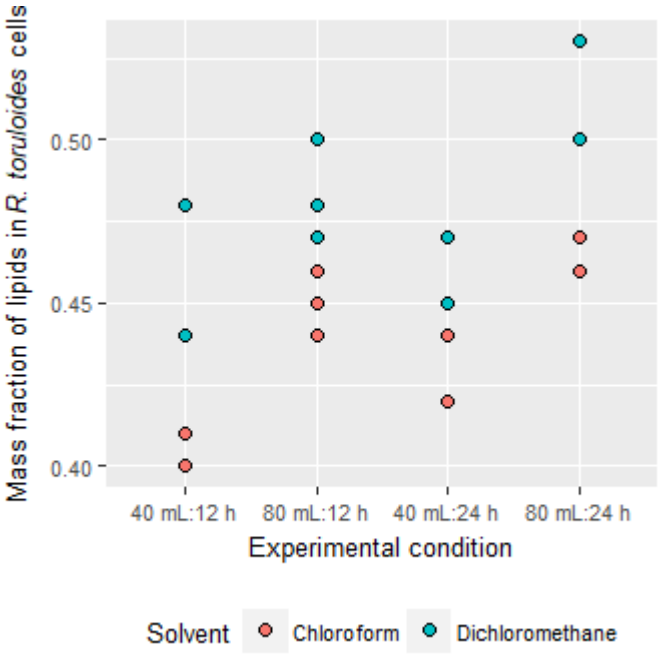
Chart 1. Parameters of the 2^3 factorial experiment

Factor	Levels	Observed p-value (99% confidence)
Type of solvent	$CHCl_3$, CH_2Cl_2	<0.001
Volume of solvent	40 mL, 80 mL	<0.001
Time of extraction	12 h, 24 h	0.004

Since all p-values were less than 0.005 (99% confidence level), all factors may have a significant impact on the response variable (mass fraction of SCO). The blockage variable showed a p-value of 0.007, indicating that the variation among blocks was not significant.

An exploratory analysis of the data is portrayed in Figure 1, where it can be seen that and volume of solvent appear to greatly influence the mass fraction outcome. Time of extraction showed little effect, although significant.

Figure 1. Variation of lipid mass fraction at each experimental condition



Moreover, the best experimental condition corresponded to 80 mL of solvent and 24 h of extraction for both solvents. Still, dichloromethane yielded higher lipid mass fractions, probably due to the occurrence of a higher saturation point compared to chloroform.

Conclusions

The results showed that dichloromethane is an alternative to chloroform in the extraction of SCO from *Rhodotorula toruloides*, with equivalent or greater extraction performance.

Acknowledgement

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[1] Cequier-Sánchez, E. et al. Dichloromethane as a Solvent for Lipid Extraction and Assessment of Lipid Classes and Fatty Acids from Samples of Different Natures. *J. Agric. Food Chem.*, **2008**, 56 (12), 4297–4303.

[2] Bonturi, N. Produção de óleo microbiano por cepas adaptadas de leveduras oleaginosas a partir de materiais hemicelulósicos visando a sua aplicação em biorrefinarias, Ph.D. Dissertation, University of Campinas, Campinas, SP, 2016.