Biotechnology for Biofuels and Bioproducts

METHODOLOGY

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Binary solvent extraction of intracellular lipids from *Rhodotorula toruloides* for cell recycling

Jingyi Song[1], Rasool Kamal[1], Yadong Chu[1,4], Shiyu Liang[1,3], Zongbao K. Zhao[1,3] and Qitian Huang[1,2,4*]

Abstract

Background Microbial lipid extraction is a critical process in the production of biofuels and other valuable chemicals from oleaginous microorganisms. The process involves the separation of lipids from microbial cells. Given the complexity of microbial cell walls and the demand for efficient and environmentally friendly extraction methods, further research is still needed in this area. This study aims to pursue the extraction of intracellular lipids from oleaginous yeasts using inexpensive solvents, without disrupting the cells and even maintaining a certain level of cell viability.

Results The study used fresh fermentation broth of *Rhodotorula toruloides* as the lipid extraction target and employed a binary solvent of methyl tert-butyl ether (MTBE) and n-hexane for lipid extraction. The effects of extraction time and solvent ratio on cell viability, lipid extraction efficiency, and fatty acid composition were analyzed. Conditions that balanced lipid yield and cell survival were selected for lipid extraction.

Specifically, using a binary solvent (with 40% MTBE) to extract an equal volume of *R. toruloides* fermentation broth achieved a total lipid extraction rate of 60%, while maintaining a 5% cell survival rate (the surviving cells served as the seed for the second round of lipid production). After separating the solvent phase and supplementing the lipid-extracted cells with carbon sources and a small amount of nitrogen sources, the cells gradually regained biomass and produced lipids. Repeating this "gentle" extraction on surviving and regrown cells and adding carbon and nitrogen sources can enable a second round of growth and lipid production in these cells.

Conclusions This is an interesting finding that may potentially encompass the extraction mechanisms of polar/nonpolar solvents and the phenomenon of yeast autophagy. This method does not require the destruction of the cell wall of oleaginous yeast. The separation after extraction is simple, and both the cells and solvents can be recycled. It provides a possible approach for simultaneous fermentation and lipid extraction.

Keywords Oleaginous microorganisms, Lipid extraction, MBTE, Cell recycling

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Introduction

Oleaginous microorganisms are an alternative source of oils, distinct from plants, animals, and petroleum. They produce what is known as single cell oils (SCO), which can be used in biofuels, pharmaceuticals, and other industries. These microorganisms include certain yeasts, bacteria, and algae [1]. With their short production cycles, high lipid yields, genetic tractability, and industrial scalability, oleaginous microorganisms are considered ideal substitutes for fossil fuels [2].

Rhodotorula toruloides is renowned for its broad substrate spectrum, strong stress resistance, and high lipid yield. These attributes make it an ideal candidate for the production of lipids, carotenoids, and other valuable chemicals [3]. The research has shown that under nitrogen-limited conditions, *R. toruloides* undergoes lipid remodeling, where phospholipids are converted into storage lipids. This process enhances the accumulation of both lipids and carotenoids [4, 5].

However, the unique cellular structures of oleaginous microorganisms present significant challenges for lipid extraction. Given the minuscule size of these microbial cells, traditional mechanical pressing methods are ineffective for recovering intracellular lipids. Moreover, the cell walls of these oleaginous microorganisms, particularly those of oleaginous yeasts, are rich in chitin and mannans. These components endow the cell walls with high mechanical strength, thickness, and density, further complicating the extraction of lipids from microbial cells [6, 7]. Therefore, before solvent extraction, pretreatment or cell disruption methods, such as mechanical (puffing, bead milling, ultrasonication, homogenization, microwave) and nonmechanical (enzymatic, acid, alkaline digestion, osmotic shock) approaches are crucial for the effective recovery of lipids from oleaginous microorganisms [8]. After pretreatment, the increased accessibility of intracellular lipids makes the subsequent extraction of lipids from the pretreated material using organic solvents much more efficient and straightforward. However, inevitably, the disrupted cells can introduce impurities into the solvent phase, and the pretreatment process also involves a significant amount of energy consumption.

The current lipid extraction technologies have also seen the development of novel green solvent-based methods. For example, the use of dimethyl carbonate (DMC) and supercritical CO_2, combined with deep eutectic solvents (DESs) and microwave pretreatment, has been shown to enhance lipid extraction [9]. Subcritical dimethyl ether (DME) [10] and switchable polarity solvents, such as dipropylamine (DPA) after CO_2 treatment [11, 12], enable direct lipid extraction from wet biomass without the need for pretreatment. Meanwhile, highly economical extraction methods have also been explored, such as milk lipids without affecting the growth of the microalgae *Botryococcus braun*i using n-hexane [13].

This study presents a method for lipid extraction from *R. toruloides* fermentation broth using a binary solvent system of methyl tert-butyl ether (MTBE) and n-hexane. The method involves separating the viable cells that remain after lipid extraction, replenishing the medium, and then conducting lipid production and extraction again, repeating this process for two cycles. By investigating the solvent ratio and extraction duration, the optimal extraction strategy was determined. This approach provides a practical method for lipid recovery and resource recycling that can help reduce the cost of microbial lipid production.

Materials and methods

Microorganism and solvents

R. toruloides CGMCC 2.1389 was obtained from the China General Microbiological Culture Collection Center. Methyl tert-butyl ether (MTBE), n-hexane and chloroform were purchased locally and were of analytical reagent grade.

Cultivation conditions

The initial seed (fresh culture of *R. toruloides*) was inoculated and enriched in yeast extract peptone dextrose (YEPD) medium, cultured at 30 °C with shaking at 200 rpm for 24 h. The enriched culture was inoculated into nitrogen-limited medium at a 10% (v/v) inoculum size for lipid production and cultured at 30 °C with shaking at 200 rpm until complete glucose consumption.

YEPD medium: glucose, 20 g/L; yeast extract, 10 g/L; and peptone, 20 g/L.

Nitrogen-limited medium: glucose, 50 g/L; $(NH_4)_2SO_4$, 0.1 g/L; yeast extract, 0.75 g/L; $MgSO_4{\cdot}7H_2O$, 1.5 g/L; and trace elements solution, 10 mL/L.

Trace elements solution: $CaCl_2{\cdot}2H_2O$ 4 g/L, $FeSO_4{\cdot}7H_2O$ 0.055 g/L, Citric acid monohydrate 0.52 g/L, $ZnSO_4{\cdot}7H_2O$ 0.1 g/L, $MnSO_4{\cdot}H_2O$ 0.076 g/L, 18 M H_2SO_4 100 µL/L.

pH adjustment: 2-(N-Morpholino) ethanesulfonic acid hydrate (MES) buffer was added at 50 mmol/L to maintain the pH at 6.0.

Take 50 mL of fermentation broth for binary solvent lipid extraction. After extraction, take 30 mL of the lower layer of the spent broth and supplement nutrients according to the following strategy:

Nitrogen-limited (Nl) supplemented: 250 g/L glucose solution, 10 mL; 39 g/L MES buffer solution, 5 mL; sugar-free nitrogen-limited medium solution (yeast extract, 7.5 g/L; $(NH_4)_2SO_4$, 1 g/L; $MgSO_4{\cdot}7H_2O$, 6 g/L; 40 mL/L trace element solution), 5 mL; total 50 mL system.

Nitrogen-rich (Nr) supplemented: An additional 5 mL of YEPD medium and other nutrient supplements consistent with Nl were added; a total of 55 mL of the system was used.

Lipid extraction method

Extraction of total lipids

The total lipids were extracted by acid–heating extraction (AHE) method. For each gram of dry cells, 6 mL of 4 M HCl was added and hydrolyzed at 78 °C for 1 h. After cooling, an equal volume of chloroform and methanol was added, vortexed for 3 min, and then centrifuged at 8000 r/min for 5 min to separate the organic phase. An equal volume of chloroform was added again for a second extraction, and the organic phases were separated and combined. The combined organic phase was washed with an equal volume of 0.1% NaCl solution, and the resulting extract was purified and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the lipids were dried to constant weight at 105 °C and weighed. The lipid content was expressed as grams of lipids per liter of fermentation broth and was used as the standard for evaluating the lipid extraction efficiency of other methods.

Optimization of binary solvent conditions

15 mL of fermentation broth was taken at 0 h, 24 h, 48 h, 72 h, 96 h, and 120 h, respectively. Then, 15 mL of MTBE/hexane binary mixture containing 0%, 20%, 40%, 60%, 80%, and 100% MTBE was added to each sample. The extraction was carried out at 200 rpm for 30 min, 60 min, and 90 min, respectively. The solvent phase was separated by centrifugation, and moisture and protein impurities were removed using anhydrous sodium sulfate. The solvent was evaporated under reduced pressure, and the lipids were dried to constant weight. Three replicates were set for each condition.

Lipid extraction during the re-fermentation process of lipid-extracted cells

Lipid extraction was carried out using the MTBE-hexane binary solvent (40% MTBE), following the extraction and purification procedures described in"Optimization of binary solvent conditions"section.

Analytical methods

Determination of culture parameters

The cell mass was determined via the dry weight method. The glucose content of the culture broth was measured via a biosensing analyser. The OD_{600} value was obtained by measuring the absorbance at 600 nm via a UV–Vis spectrophotometer.

Determination of total lipid content and analysis of fatty acid composition

The fatty acid composition of lipids was analyzed using gas chromatography–mass spectrometry (GC–MS) according to a previously described method [14].

Staining and observation methods

Examination of intracellular lipid droplets

9-(diethylamino) benzo[α]phenoxazin-5(5H)-one (Nile red), a lipophilic dye, can stain lipid droplets inside cells red. Nile red staining solution at a final concentration of 1 μg/mL was added to the cultures, which were subsequently stained in the dark for 5 min [15] and then observed in the RFP mode of the microscopic cell imaging system.

Examination of cell survival and the cell membrane

Propidium iodide (PI), a DNA dye, can stain cells that have lost membrane integrity and can also be used to assess cell viability. PI staining solution at a final concentration of 1 μg/mL was added to the cultures, which were then stained in the dark for 15 min and observed in the RFP mode of the microscopic cell imaging system.

In addition, the streak plate method and spread plate method were employed to assess the viability of the cells.

Results and discussion

Results and mechanism of solvent treatment on R. toruloides

Chloroform is commonly used for lipid extraction from oleaginous yeast cells, but it is somewhat toxic. MTBE is a greener solvent that has been used for lipid extraction in lipidomics analysis [16]. In addition, n-hexane has been used to extract lipids during the cultivation of microalgal cells [13]. To test whether *R. toruloides* cells could tolerate the extraction process with these solvents, cells treated with the three solvents (with ethanol as a polar solvent control) were collected and stained with PI and Nile Red.

The results showed that cells treated with ethanol, MTBE, and chloroform were all stained by PI, indicating cell death, while cells in the n-hexane group remained viable. The light field view in Fig. 1 clearly showed that chloroform and MTBE extracted the lipids from *R. toruloides* cells, leaving them as "empty shells," whereas the polar solvent ethanol failed to extract intracellular lipids. Nile Red staining revealed that only the n-hexane group retained intact lipid droplet structures, while other groups showed organic solvents mixed with intracellular lipids being stained. Chloroform exhibited significant

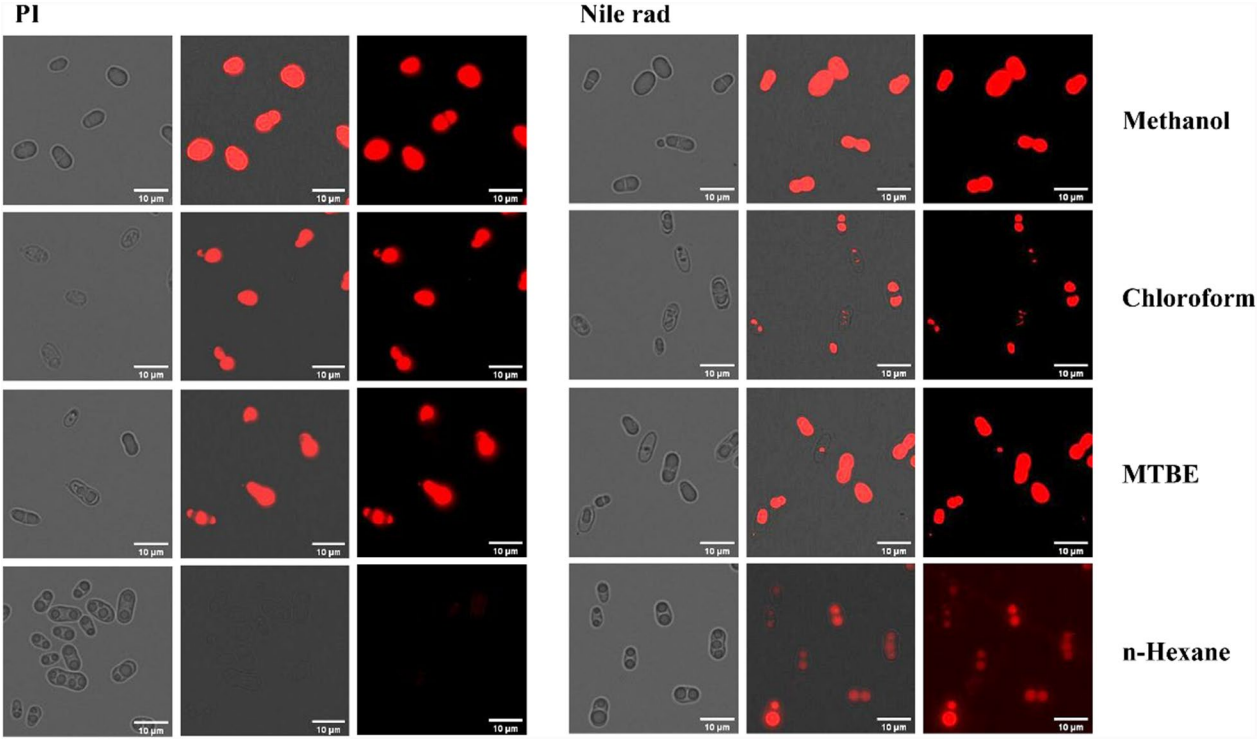

Fig. 1 Staining of cells after lipid extraction with PI and Nile Red using solvents (the figure for the light field view, light and dark field superimposed view, dark field view)

lipid extraction efficiency in this experiment, the lipid droplets inside the cells have almost disappeared.

Ethanol is miscible with water, while chloroform and MTBE are sparingly soluble in water (with solubilities of 0.8 g/L and 15 g/L, respectively). In contrast, n-hexane has an extremely low solubility in water and is virtually insoluble. The extraction mechanisms of these three types of solvents can be illustrated in Fig. 2. The pathway for solvent mass transfer to intracellular lipids can be summarized as follows: solvent phase → cell wall → cytoplasmic membrane → cytoplasmic matrix → lipid droplet membrane → lipids. For polar solvents that are soluble in water, the mass transfer through the cell wall, cytoplasmic membrane, cytoplasmic matrix, and lipid droplet membrane is relatively straightforward, with the main resistance being the dissolution of lipids. For weak-polar solvents, both the cell membrane and intracellular lipids can be dissolved, facilitating the extraction of lipids from within the cells. However, different solvents will exhibit varying dissolution capabilities. For nonpolar solvents, the water in the cell wall pores, the cytoplasmic membrane with its hydrophilic ends facing outward, the cytoplasmic matrix, and the lipid droplet membrane all pose significant barriers to mass transfer. As a result, even if nonpolar solvents have good compatibility with lipids, they are unable to extract intracellular lipids effectively.

Exploration of lipid extraction methods balancing lipid recovery and cell viability

Based on the above results, MTBE can be attempted as a bridge for the entry and exit of cells, like Figure S1, while n-hexane can serve as a storage solvent to accommodate lipids. Generally, the oil-producing cultivation of *R. toruloides* reaches the fermentation endpoint at 96–120 h. However, there are differences in lipid content, cell wall composition, and thickness at different cultivation time [17]. Therefore, fermentation broths of *R. toruloides* cultured for different durations were used as extraction materials, and binary solvents of MTBE/n-hexane at different ratios were employed as oil-extraction solvents to investigate the effects of various factors on lipid extraction.

As shown in Fig. 3, except for the cultivation duration of 24 h, the lipid extraction capacity was significantly influenced by the MTBE ratio during cultivation durations of 48–120 h, with an increased MTBE ratio enhancing the lipid extraction capacity.

In addition, as the cultivation time increased, the intracellular oil content rose, and the lipid extraction efficiency significantly improved. The accumulation of lipids enlarged the cell volume and narrowed the gap between the lipid droplets and the cell wall, thereby shortening the mass transfer pathway of the solvent. As shown in Figure S2, the

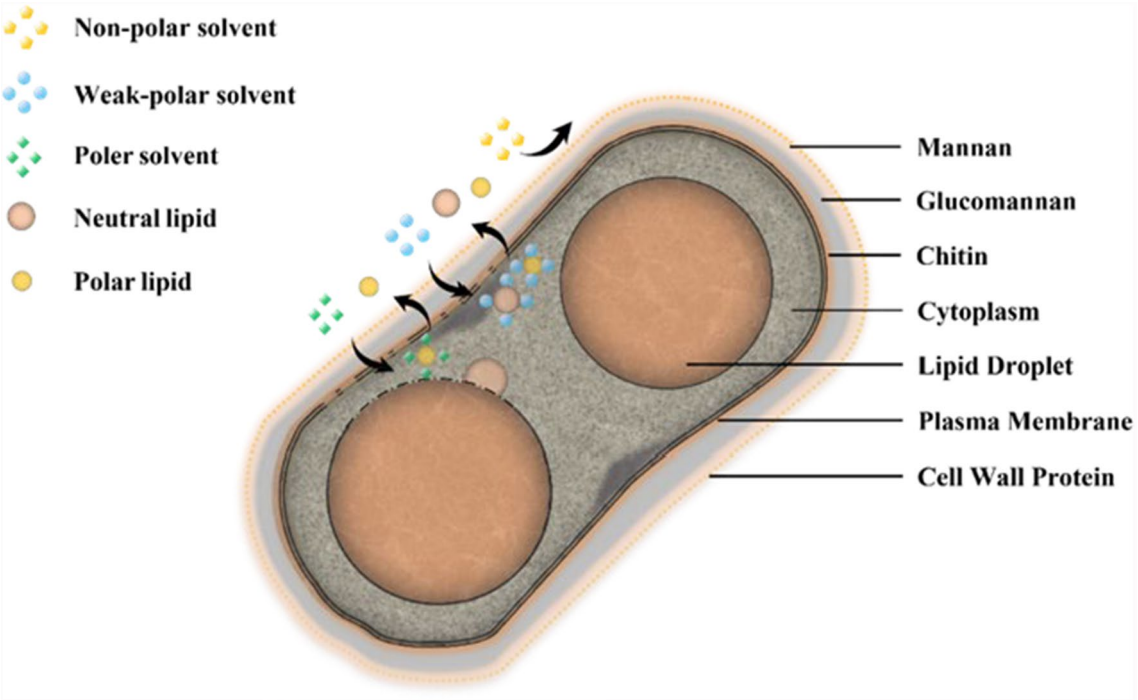

Fig. 2 Mechanism of oil extraction with three types of solvents

microscopic images of intracellular lipids at 48 h and 120 h correspond to lipid contents of 37% and 65%, respectively, indicating that the higher the intracellular lipid content, the better the extraction effect. The extraction efficiency of the binary solvent increased with the rise in MTBE content, while a high content of n-hexane tended to play a "cell-protecting" role.

As illustrated in Figure S3, when using a binary solvent with a 40% MTBE ratio, some cells survived after extraction. It can be inferred that cells in good condition with lower lipid content might be less affected by the binary solvent, becoming "survivors" after lipid extraction and resuming growth through self-repair. The lipid extraction data were summarized in Fig. 4, which shows that during oil extraction from the 120-h fermentation broth, the most significant change in oil extraction capacity occurred when the MTBE ratio was increased from 20 to 40%. When compared with the methods in Table S1, the lipid recovery rate and process complexity have been reduced. Therefore, the optimal solvent ratio was selected as a binary mixture of 40% MTBE/n-hexane.

The bacterial suspensions before and after lipid extraction were counted, and the survival rate of the bacterial cells was calculated using the following formula resulting in a survival rate of 5.34%.

$$\boldsymbol{suevival\%} = \frac{\boldsymbol{N}_1 \times \boldsymbol{D}_1 \div 100\boldsymbol{\mu L}}{\boldsymbol{N}_2 \times \boldsymbol{D}_2 \div 100\boldsymbol{\mu L}}$$

N_1: Number of strains growing after lipid extraction
N_2: Number of strains growing before lipid extraction
D: Dilution times

Within the same cultivation period, the latter was smaller than the former (as shown in Figure S4), indicating that the growth rate of the strain had slowed down after lipid extraction and required a certain period of recovery.

Figure 5 shows the fatty acid composition of lipids extracted using different ratios of binary solvents. The fatty acid composition of lipids extracted with 100% n-hexane differs greatly from that of lipids extracted with MTBE added solvents. This is because n-hexane has difficulty penetrating cell membranes to extract intracellular lipids, so it can only extract lipids from the cell exterior or a few ruptured cells, resulting in extremely low lipid extraction rates and significant differences in lipid composition as compared to the AHE method. After adding MTBE to form a binary solvent, the fatty acid composition of the obtained lipids was C16:0≈25%, C18:0≈13%, C18:1≈50%, C18:2≈10%, which is comparable to that of AHE. This indicates that MTBE plays a major role in lipid extraction and extracts intracellular lipids indiscriminately.

Recycling of surviving cells after lipid extraction

Based on the above observations, we designed a new lipid production process in which a carbon source is

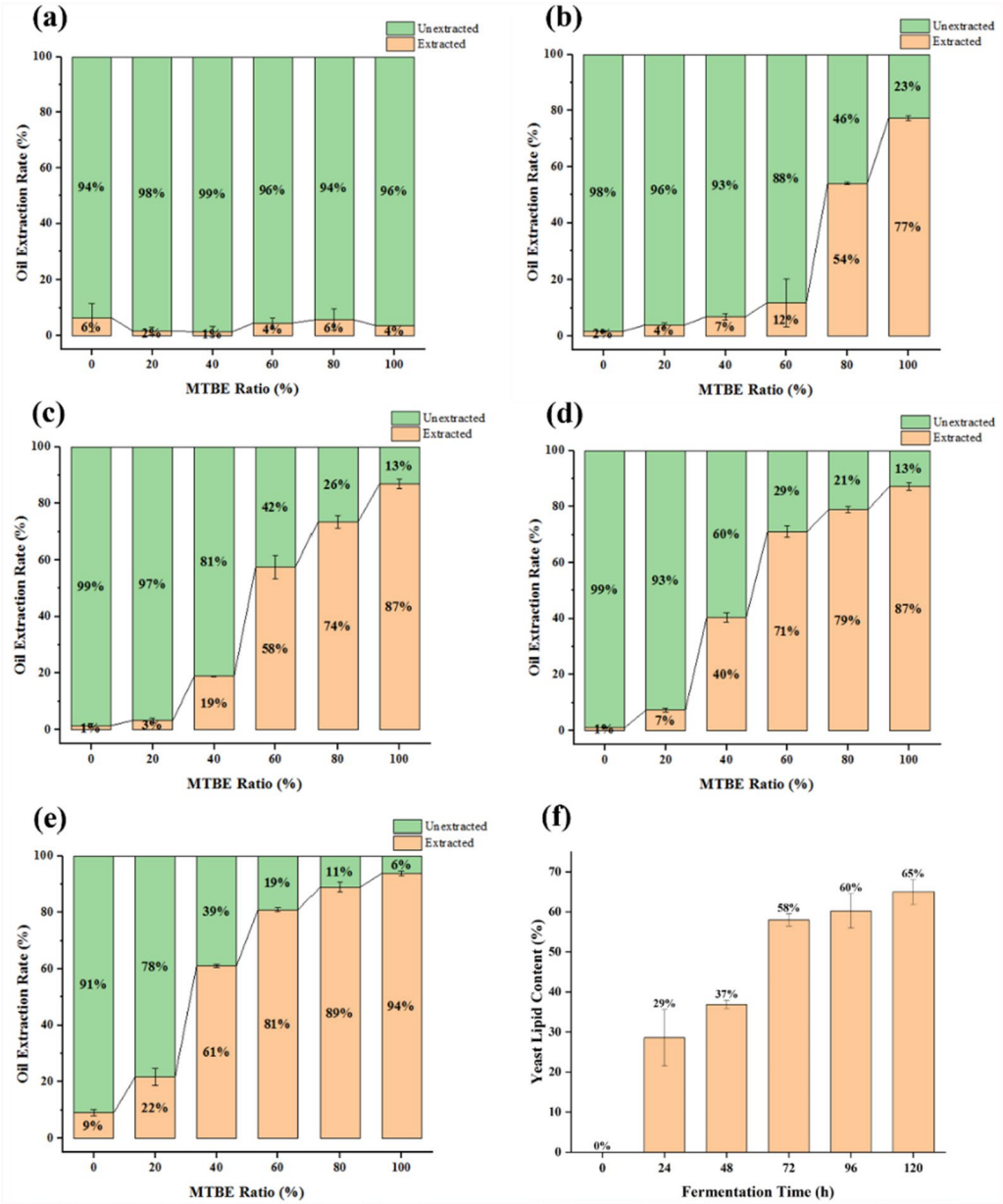

Fig. 3 Lipid extraction with different ratios of MTBE/n-hexane binary solvents at different periods ((**a**): 24 h, (**b**): 48 h, (**c**): 72 h, (**d**): 96 h, (**e**): 120 h; (**f**) the lipid content during fermentation)

added to the spent cultures of *R. toruloides* after in situ lipid extraction with a 40% MTBE/n-hexane solvent for the subsequent production of lipids. Figure 6 shows the differences in cell growth, sugar consumption, and lipid production under nitrogen-limited (Nl) and nitrogen-rich (Nr) conditions due to the recycling of the spent cultures.

The average cell growth rate of the initial culture over 96 h was 12.75 OD/d, and the sugar consumption rate was 11.38 g/(L·d). Because a large amount of lipids was removed and only 60% of the spent culture was transferred to initiate a new round of cultivation after the addition of glucose and nitrogen sources, the OD value of the culture broth decreased at the beginning of each round. However, cell growth and glucose consumption resumed within 1 day (Fig. 6a, b). For the Nl trials, a small amount of nitrogen source was added, and cell growth and glucose utilization increased rapidly after the lag phase. The average cell growth rate was 5.98 OD/d, and the sugar consumption rate was 7.18 g/

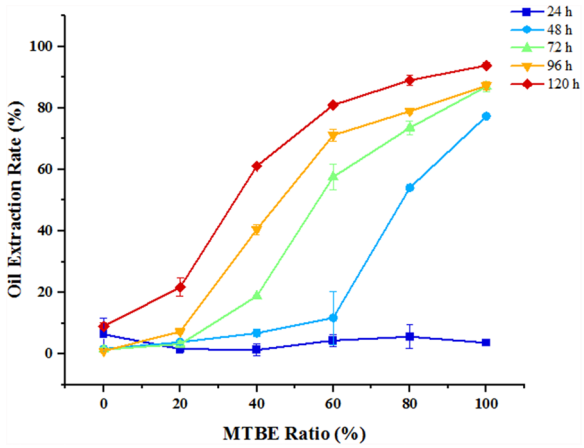

Fig. 4 Lipid extraction rates of different ratios of MTBE/hexane binary solvents at different incubation times

(L·d) in the first round of lipid production. In the second round, the average rates of cell growth and sugar consumption decreased to 4.06 OD/d and 6.00 g/(L·d), respectively. For the Nr trials with additional YEPD supplementation, cell growth and glucose utilization were faster than those in the Nl trials, with average cell growth and sugar consumption rates of 6.55 OD/d and 7.94 g/(L·d) in the first round and 6.05 OD/d and 7.80 g/(L·d), respectively, in the second round. Although the cell growth and sugar consumption were faster in the Nr trials, the final lipid yield was 0.108 g/g, which was lower than the 0.131 g/g obtained in the Nl trials (Fig. 6 c).

The process shown in Fig. 6 is different from the previous processes used for lipid production. In the repeated fed-batch fermentation process, a small portion of the mature culture containing lipid-rich cells is left in the reactor as an inoculum to initiate new lipid production upon the addition of fresh media [18], and the proportion of dead cells has decreased. (as shown in Figure S5). Notably, the spent cell mass after lipid extraction has been chemically hydrolyzed, and the resulting hydrolysates have been used as nutrients for lipid production [19]. In this study, most of the culture broth (including cells) was recycled for the next round of cultivation. The surviving cells were used as seeds, and the spent cells were used as slow-release nutrients, retaining both organic matter and mineral nutrients. This likely achieved in situ utilization of waste and provided a material basis for lipid synthesis after the replenishment of carbon sources. From an economic perspective, the 5% viable cell recovery and 60% extraction rate may not give biodiesel production a significant economic advantage, but the 5% cell survival supports cell recycling and normal lipid production. This process allows cells to be used in situ for further fermentation without separation. Dead cells can be broken down by hydrolytic enzymes and taken up by living cells as nutrients for growth during yeast cultivation [20]. This study presents a more efficient recycling process for wastewater, nutrients, and cell

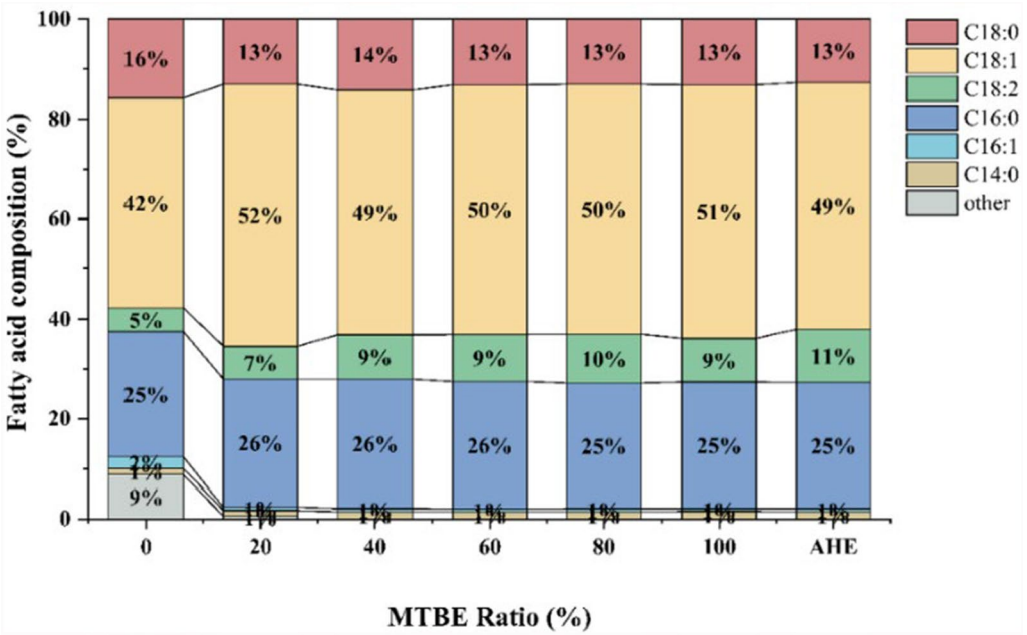

Fig. 5 Fatty acid composition of lipids extracted using different ratios of combined solvents (120 h fermentation culture;0% ~ 100% represents the percentage of MTBE in the combined solvent)

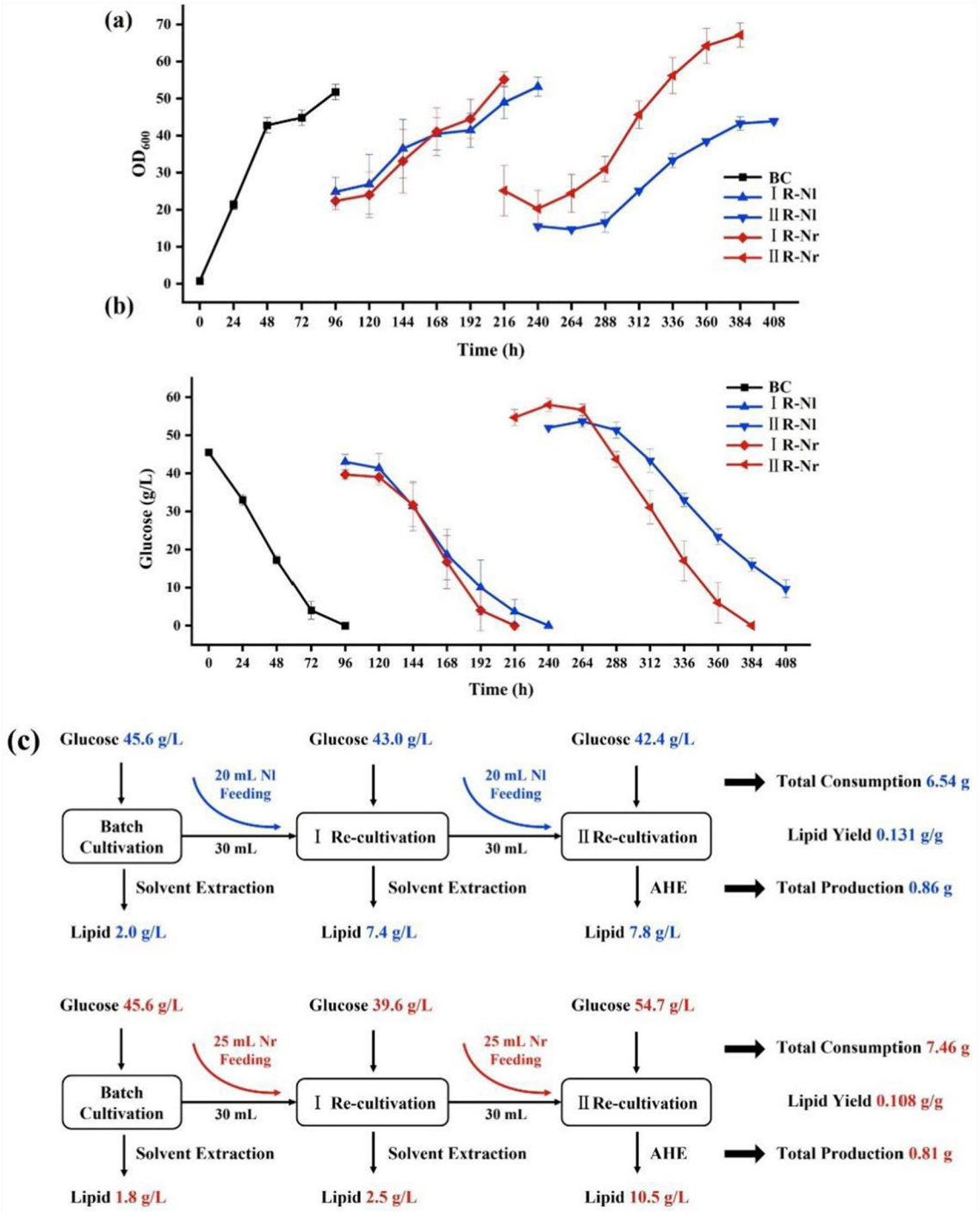

Fig. 6 Results of the cultivation–extraction–recultivation process in which *R. toruloides* was used as the lipid producer. ((**a**) Cell growth profiles; (**b**) glucose consumption profiles; (**c**) mass flow of the cultivation–extraction–recultivation process; blue: Nl feeding; red: Nr feeding))

mass in microbial lipid technology, offering new ideas for future continuous operation.

Conclusions

We have demonstrated that under mild conditions, a mixture of MTBE and n-hexane can effectively extract lipids directly from the fermentation broth of *R. toruloides* without cell disruption, achieving a 60% lipid extraction rate while maintaining a 5% cell survival rate. The surviving cells present in the spent fermentation broth regrown and produced lipids upon supplementation with fresh carbon and nitrogen sources which enables a cyclic process of lipid extraction and cell regrowth. By combining polar and nonpolar solvents for lipid extraction, this new approach exempts energy-intensive thermochemical cell disruption, reduces waste generation by recycling fermentation broth and solvents, and allows semi-continuous lipid production. The findings provide a promising foundation for further optimization and scaling of simultaneous fermentation and lipid extraction processes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13068-025-02655-0.

Supplementary materials 1

Author contributions

Jingyi Song designed and performed this research; Rasool Kamal and Shiyu Liang helped Jing yi Song perform this research; Chu Yadong analyzed data; Qitian Huang and Zongbao Zhao gave funding support and advices.

Funding

The work was financially supported by the National Key Research and Development Program of China (No. 2021YFA0910603), National Natural Science Foundation of China (No. 22238010 and 22208340), Science and Technology Bureau of Dalian City (No. 2021RT04), the Natural Science Foundation of Liaoning Province (2024-BSBA-29).

Availability of data and materials

The data collected upon which this article is based upon are all included in this manuscript and the additional files associated with it. No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

No animals or human subjects were used in the above research.

Consent for publication

Our manuscript does not contain any individual data in any form.

Competing interests

The authors declare no competing interests.

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Received: 24 January 2025 Accepted: 30 April 2025
Published online: 12 May 2025

References

1. Meng X, Yang J, Xu X, Zhang L, Nie Q, Xian M. Biodiesel production from oleaginous microorganisms. Renewable Energy. 2009;34:1–5.
2. Othman MF, Adam A, Najafi G, Mamat R. Green fuel as alternative fuel for diesel engine: a review. Renew Sustain Energy Rev. 2017;80:694–709.
3. Xylose Metabolism and the Effect of Oxidative Stress on Lipid and Carotenoid Production in *Rhodotorula toruloides*: Insights for Future Biorefinery-PubMed. https://pubmed.ncbi.nlm.nih.gov/32974324/. Accessed 19 Mar 2025.
4. Mishra S, Deewan A, Zhao H, Rao CV. Nitrogen starvation causes lipid remodeling in *Rhodotorula toruloides*. Microb Cell Fact. 2024;23:1–14.
5. Compositional profiles of *Rhodosporidium toruloides* cells under nutrient limitation | Applied Microbiology and Biotechnology. https://link.springer.com/article/https://doi.org/10.1007/s00253-017-8157-0. Accessed 19 Mar 2025.
6. Khot M, Raut G, Ghosh D, Alarcón-Vivero M, Contreras D, Ravikumar A. Lipid recovery from oleaginous yeasts: perspectives and challenges for industrial applications. Fuel. 2020;259: 116292.
7. Jaussaud A, Lupette J, Salvaing J, Jouhet J, Bastien O, Gromova M, et al. Stepwise biogenesis of subpopulations of lipid droplets in nitrogen starved *Phaeodactylum tricornutum* cells. Front Plant Sci. 2020;11:48.
8. Zainuddin MF, Fai CK, Ariff AB, Rios-Solis L, Halim M. Current pretreatment/cell disruption and extraction methods used to improve intracellular lipid recovery from oleaginous yeasts. Microorganisms. 2021;9:251.
9. Tommasi E, Cravotto G, Galletti P, Grillo G, Mazzotti M, Sacchetti G, et al. Enhanced and selective lipid extraction from the microalga *P. tricornutum* by dimethyl carbonate and supercritical CO2 using deep eutectic solvents and microwaves as pretreatment. ACS Sustainable Chem Eng. 2017;5:8316–22.
10. Wang Q, Oshita K, Takaoka M. Effective lipid extraction from undewatered microalgae liquid using subcritical dimethyl ether. Biotechnol Biofuels. 2021;14:17.
11. Yook SD, Kim J, Woo HM, Um Y, Lee S-M. Efficient lipid extraction from the oleaginous yeast *Yarrowia lipolytica* using switchable solvents. Renewable Energy. 2019;132:61–7.
12. Choi OK, Lee JW. CO_2-triggered switchable solvent for lipid extraction from microalgal biomass. Sci Total Environ. 2022;819: 153084.
13. Jackson BA, Bahri PA, Moheimani NR. Repetitive non-destructive milking of hydrocarbons from *Botryococcus braunii*. Renew Sustain Energy Rev. 2017;79:1229–40.
14. Kamal R, Huang Q, Li Q, Chu Y, Yu X, Limtong S, et al. Conversion of *Arthrospira platensis* biomass into microbial lipids by the oleaginous yeast *Cryptococcus curvatus*. ACS Sustain Chem Eng. 2021;9:11011–21.
15. Chung T-Y, Kuo C-Y, Lin W-J, Wang W-L, Chou J-Y. Indole-3-acetic-acid-induced phenotypic plasticity in *Desmodesmus algae*. Sci Rep. 2018;8:10270.
16. Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J Lipid Res. 2008;49:1137.
17. Li Y. Lipid production by *Rhodosporidium toruloides*. Dalian University of Technology; 2007.
18. Zhao X, Hu C, Wu S, Shen H, Zhao ZK. Lipid production by *Rhodosporidium toruloides* Y4 using different substrate feeding strategies. J Ind Microbiol Biotechnol. 2011;38:627–32.
19. Yang X, Jin G, Gong Z, Shen H, Bai F, Zhao ZK. Recycling microbial lipid production wastes to cultivate oleaginous yeasts. Biores Technol. 2015;175:91–6.
20. Carmona-Gutierrez D, Ruckenstuhl C, Bauer MA, Eisenberg T, Büttner S, Madeo F. Cell death in yeast: growing applications of a dying buddy. Cell Death Differ. 2010;17:733–4.

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