Journal of Biotechnology 366 (2023) 10–18

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Fermentation performance of oleaginous yeasts on *Eucommia ulmoides Oliver* hydrolysate: Impacts of the mixed strains fermentation

Ruiling Gao [a,b,c,d], Hairong Zhang [a,b,c,d], Lian Xiong [a,b,c,d], Hailong Li [a,b,c,d], Xuefang Chen [a,b,c,d], Mengkun Wang [a,b,c,d], Xinde Chen [a,b,c,d,*]

[a] Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, People's Republic of China
[b] CAS Key Laboratory of Renewable Energy, Guangzhou 510640, People's Republic of China
[c] Guangdong Provincial Key Laboratory of New and Renewable Energy Research and Development, Guangzhou 510640, People's Republic of China
[d] R&D Center of Xuyi Attapulgite Applied Technology, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Xuyi 211700, People's Republic of China

ARTICLE INFO

Keywords:
Oleaginous yeast
Mixed strains fermentation
Microbial lipid
Yeast polysaccharide
Eucommia ulmoides Oliver
Hydrolysates

ABSTRACT

This present study mainly focused on the investigation and optimization of the fermentation performance of oleaginous yeasts on *Eucommia ulmoides Oliver* hydrolysate (EUOH), which contains abundant and diverse sugars. More importantly, the impacts of the mixed strains fermentation compared with the single strain fermentation were analyzed and evaluated, through systematic investigations of substrate metabolism, cell growth, polysaccharide and lipid production, COD and ammonia-nitrogen removals. It was found that the mixed strains fermentation could effectively promote a more comprehensive and thorough utilization of the various sugars in EUOH, greatly improve COD removal effect, biomass and yeast polysaccharide production, but could not significantly improve the overall lipid content and ammonia nitrogen removal effect. In this study, when the two strains with the highest lipid content (i.e. *L. starkeyi* and *R. toruloides*) were mixed-cultured, the maximum lipid yield of 3.82 g/L was achieved, and the yeast polysaccharide yield, COD and ammonia-nitrogen removal rates of the fermentation (LS+RT) were 1.64 g/L, 67.4% and 74.9% respectively. When the strain with the highest polysaccharide content (i.e. *R. toruloides*) was mixed-cultured with the strains with strong growth activity (i.e. *T. cutaneum* and *T. dermatis*), a large amount of yeast polysaccharides could be obtained, which were 2.33 g/L (RT+TC) and 2.38 g/L (RT+TD) respectively. And the lipid yield, COD and ammonia-nitrogen removal rates of the fermentation (RT+TC), (RT+TD) were 3.09 g/L, 77.7%, 81.4% and 2.54 g/L, 74.9%, 80.4%, respectively.

1. Introduction

Eucommia ulmoides gum (EUG), a trans-1, 4-polyisoprene natural polymer, is an important natural biomass rubber that can be used as elastic materials, thermoplastic materials, shape-memory materials, etc (Kang et al., 2018; Sun et al., 2018; Xia et al., 2019). EUG is usually extracted from the leaves, bark and samara of *Eucommia ulmoides Oliver* (EUO), a widely distributed valuable economic plant in China (Yang et al., 2020). Different from natural rubber which is composed of cis-1, 4-polyisoprene and can be gathered from the sap of plants, the extraction of EUG from EUO mainly includes three steps: pretreatment, extraction and purification, and pretreatment is considered the most important step that can efficiently destroy the EUG-containing cell wall and improve the yield of EUG (Qin et al., 2021). Compared with the mechanical process (Wei et al., 2019) or alkaline pretreatment and fermentation (Liu et al., 2010; Zhang et al., 2009), dilute acid hydrolysis pretreatment is gaining more and more applications because of its high efficiency and EUG yield (Qin et al., 2021; Yang et al., 2020). More importantly, dilute acid hydrolysis is a state-of-the-art chemical technology for the conversion of lignocellulosic biomass to fermentable sugars and other bio-based platform compounds (Besson et al., 2014; Huang et al., 2019; Xu et al., 2016). Thus, the wastewater produced in the pretreatment process, namely *Eucommia ulmoides Oliver* hydrolysate (EUOH), can be treated and re-utilized through microbial treatment technology, so as to further increase the added value of the EUG production and reduce environmental pollution.

As a sort of lignocellulose biomass, EUO contains a large amount of cellulose and hemicellulose, making the EUOH contain a variety of carbohydrates, mainly including glucose, xylose, arabinose, cellobiose, etc., as well as a small amount of volatile fatty acids (VFAs) dominated

* Correspondence to: No. 2 Energy Road, Tianhe District, Guangzhou 510640, China.
E-mail address: cxd_cxd@hotmail.com (X. Chen).

https://doi.org/10.1016/j.jbiotec.2023.02.009
Received 1 November 2022; Received in revised form 15 January 2023; Accepted 26 February 2023
Available online 1 March 2023

by acetic acid (Yang et al., 2020). And when organic acids (e.g. formic acid, acetic acid) are used as catalyst in the dilute acid hydrolysis process, the possible residual acids will further increase the total organic acids concentration in the EUOH. It is well known that xylose and arabinose (five-carbon sugars) usually cannot be fermented by most yeasts including native *Saccharomyces cerevisiae* strains (Cai et al., 2012). In particular, xylose, as the final hydrolysate of hemicellulose, is the most abundant pentose sugar in EUOH as well as in nature. However, the current utilization of xylose is limited to the production of feed yeast, furfural, and xylitol (Finneran and Popovic, 2018; Ghindea et al., 2010; Zhao et al., 2020). How to expand and optimize the utilization of pentoses represented by xylose is of great significance to fermentation engineering. In addition, it is reported that organic acids, which also contained in EUOH, could have certain inhibitions on the growth of microorganisms. Especially, the high concentration of acids could lead to the suspension of many fermentation processes, and even the death of microorganisms (Fei et al., 2011; Rodrigues and Pais, 2000; Royce et al., 2013). Considering the complexity and inhibition of the components of the EUOH, it is of great significance to study appropriate microbial treatment methods to realize the comprehensive and efficient utilization of organic components in EUOH.

In recent years, more and more attention has been paid to the treatment and high-value utilization of organic wastes by the lipid-producing fermentation process with oleaginous yeast (Cho and Park, 2018; Qin et al., 2017). Compared with the anaerobic fermentation process of producing biogas, ethanol, butanol, etc., lipid-producing fermentation is an aerobic fermentation process, which is much safer and easier to control. Besides, among various industrial microorganisms, oleaginous yeasts are relatively robust species and are easy to cultivate (Gao et al., 2017; Suutari et al., 1990). More importantly, oleaginous yeasts could use various types of low-cost substrates for cell growth and lipid synthesis, e.g. crude glycerol (Kamal et al., 2022), wastewater (Chen et al., 2012; Wen and Li, 2021), lignocellulose biomass hydrolysate (Broos et al., 2022; Cianchetta et al., 2022; Wells et al., 2015), hydrophobic wastes (Patel and Matsakas, 2019), etc. It has been reported that a few oleaginous yeasts could take some kinds of five-carbon sugars and organic acids as carbon source (Li et al., 2020; Yu et al., 2014; Pereira et al., 2022). In addition, besides microbial lipids, yeast cells can also be used to produce polysaccharide products, which could be used as feed additives in poultry and livestock breeding (Kogan and Kocher, 2007; Li et al., 2022; Wu et al., 2021) or even play a role in anti-infective and antitumor therapy (Kogan et al., 2008; Qamar et al., 2022; Urazgaliev et al., 1992). However, for a variety of oleaginous yeasts that have been found, the fermentation performance varies with strains, and the fermentation characteristics of the same strain based on different substrates are also different (Gao et al., 2022; Zhang et al., 2022). Thus, for a specific fermentation substrate, the key to achieve good fermentation performances is to adopt appropriate strains to construct fermentation system.

The applications of microbial mixed culture, e.g. wastewater treatment, composting, and a broad spectrum of fermentative food preparations, are the historical foundation of current biotechnology. Nowadays, a targeted assembly of microorganisms to perform concerted bio-productions is forming a new cutting edge in biotechnology. Over the past decade, the research field of defined mixed cultures has gained increased attention due to their potential for process intensification and the chance to produce unknown secondary metabolites (Finneran and Popovic, 2018; Schlembach et al., 2021). Especially, for low-cost substrates with complex components or containing inhibitors, synthetic mixed cultures or co-cultures has been reported to be one of the effective means to optimize the overall fermentation performance, because it can provide a more active, robust and stable microorganism community with comprehensive and beneficial metabolic characteristics (Cheirsilp et al., 2011; Wang et al., 2022). In recent years, the number of published studies on mixed-culture consolidated bioprocesses with low-cost substrates is increasing significantly. The products of these processes are, in many cases, acids, alcohols, lipids or enzymes for biomass pretreatment and hydrolysis (Schlembach et al., 2021, 2020; Zhao et al., 2018). In the field of microbial lipid production, more concerns have been oriented towards the co-culture systems of yeast and microalgae using low cost carbon substrates (Qin et al., 2017). Quite a few researchers have proposed various higher yield co-culture systems and analyzed the synergistic effects of oleaginous yeast and microaglae (Dias et al., 2019; Wang et al., 2022). However, the research on the mixed cultures of different oleaginous yeast is still far from enough. The existing studies on oleaginous yeasts for lipid production usually focuses on the screening of strains and the influence of substrate and fermentation conditions. Some of them adopted mixed-culture mode, but it is a pity that their discussions only stay in a simple evaluation of the overall yield, while there is still a lack of systematic and in-depth research on the specific function and influence of mixed strains fermentation compared with the single strain fermentation (Gao et al., 2022; Qin et al., 2017).

This present study mainly focused on the investigation and optimization of the fermentation performance of different oleaginous yeasts on *Eucommia ulmoides Oliver* hydrolysate (EUOH), more importantly, aims to explore and evaluate the function and influence of the mixed strains fermentation, compared with the single strain fermentation. First, the single strain fermentation performance of seven species of oleaginous yeasts on EUOH was investigated. Based on the metabolic capacity of these strains to the various sugars in EUOH and their cell growth and lipid production, four strains with different advantages were selected, including *T. cutaneum* and *T. dermatis*, which could metabolize all types of sugars in EUOH well and accumulate a large amount of biomass, and *L. starkeyi* and *R. toruloides*, which could reach a high lipid content in cells. Then, the selected strains with different advantages, in pairs with different combinations, were used for the mixed strains fermentation on EUOH. Through systematic investigations of substrate metabolism, growth and lipid production, COD and ammonia-nitrogen removals in all the mixed strains fermentations, as well as further studies on the change of yeast polysaccharide, the function and effect of the mixed strains fermentation (compared with the single strain fermentation) were analyzed and evaluated.

2. Materials and methods

2.1. Materials

Eucommia ulmoides Oliver hydrolysate (EUOH) used in this present study was collected from a dilute acid hydrolysis pretreatment process (1–2 wt% acetic acid as catalyst) of *Eucommia ulmoides Oliver* leaves conducted in the Lab. In stead of sulfuric acid, acetic acid was adopted as catalyst in the dilute acid hydrolysis pretreatment process in order to avoid the negative effect of sulfate ion on the microorganism. Besides, the possible residual acetic acids, as a sort of carbon source, could be metabolized by oleaginous yeast as well (Fei et al., 2011; Vajpeyi and Chandran, 2015). The *Eucommia ulmoides Oliver* leaves were kindly provided by the Henan Fanjie agricultural development Co. Ltd., Henan Province, China. The collected EUOH has been centrifuged at 6000 rpm for 10 min to remove the suspended solids and the liquid supernatant was filtered through a 0.45 μm membrane before used as culture medium. The properties of the EUOH (after centrifugation and filtration) were shown in Table 1, and the detailed hydrolysis process of EUO leaves was described as below.

The EUO leaves were dried in a vacuum oven at 80 °C to constant weight. Without crushing, EUO leaves (50 g) and 1–2 wt% acetic acid solution (300 g) were loaded into a 500 mL stainless steel reactor. The hydrolysis of EUO leaf was conducted at 90 ℃ for 1 h under constant stirring. After the reaction, the resulting product was filtered, and the liquid part was collected as the substrate for this present study.

Table 1
Characteristics of the *Eucommia ulmoides Oliver* hydrolysate (EUOH) used as the fermentation substrate.

Item	Concentration (g/L)
SCOD	51.4 ± 1.9
Ammonia-nitrogen	1.8 ± 0.1
Total sugars	24.6 ± 2.3
Xylose	10.4 ± 1.1
Glucose	9.6 ± 1.5
Arabinose	3.3 ± 0.2
Cellobiose	1.3 ± 0.1
Total VFAs	2.6 ± 0.4
Acetic acid	1.9 ± 0.2
Formic acid	0.15 ± 0.03
Propionic acid	0.25 ± 0.08
Butyric acid	0.27 ± 0.10
Levulinic acid	N.D.
Furfural	N.D.
5-HMF	0.5 ± 0.2

SCOD: soluble chemical oxygen demand; VFAs: volatile fatty acids; 5-HMF: 5-Hydroxymethylfurfural; N.D.: not detected.

2.2. Strains and inoculum preparation

Oleaginous yeasts *Cryptococcus albidus* (CICC 31008), *Lipomyces starkeyi* (CICC 1809), *Rhodotorula glutinis* (ATCC 15125), *Rhodosporidium toruloides* (CICC 32489), *Trichosporon cutaneum* (BMZ 125173), *Trichosporon dermatis* (CICC 32903) and *Yarrowia lipolytica* (CICC 31596) were used for the fermentation in this study. For strains preservation, these yeasts were maintained at 4 °C on YPD agar slants (20 g/L agar powder) and subcultured bimonthly. The YPD medium contained 20 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract.

For the pre-culture of the seed cells, a loopfull of yeast cells was inoculated in 50 mL of YPD medium in a 250 mL flask and then incubated in a rotary shaker at 160 rpm and 28 °C for 24 h. The resulting cultures were re-inoculated in the YPD medium at a ratio of 10% (v/v) and cultivated under the same conditions for 24 h, during which the optical density of cells at 600 nm (G6860A Agilent Cary 60 UV–Vis spectrophotometer) attained 1.8–2.0, and then used as seed culture. All the equipment and solutions for microbial cultivation were steam-sterilized by autoclaving at 121 °C for 20 min

2.3. Cultures of oleaginous yeasts on EUOH

Both of the single strain fermentation and the mixed strains fermentation were studied via batch cultures performed in 250-mL Erlenmeyer flasks containing 100 mL of fermentation medium. The EUOH collected from the dilute acid hydrolysis pretreatment process of EUO leaves was centrifuged at 6000 rpm for 10 min to remove the suspended solids. The liquid supernatant was filtered through a 0.45 μm membrane before used as culture medium and no other nutrients were added.

Prior to the addition of the inoculum, the initial pH of fermentation medium was adjusted to 6.5 ± 0.1 using 3 M NaOH solution, and was sterilized at 121 °C for 20 min. After cooling to room temperature, the seed culture medium was inoculated in the fermentation medium at a total ratio of 10% (v/v). That is, for mixed cultures of two different strains (i.e. the mixed strains fermentation), the inoculation rate of seed culture medium of each strain was 5% (v/v). All batch cultures were incubated in a rotary shaker at 160 rpm and 28 °C for 120 h. During the course of fermentation, 5 mL samples were collected at regular intervals (24 h) for cell growth, residual sugars and acids analysis. At the end of fermentation, biomass was harvested for lipid extraction, yeast polysaccharide extraction and composition analysis. All the experiments were carried out in duplicate.

2.4. Analytical method

Sugars and organic acids in the fermentation broth were analyzed by HPLC (Waters 2685 systems, Waters Corp., USA), with a RI detector (Waters 2414), and on Aminex HPX-87 H column (300 mm × 7.8 mm, Bio Rad Corp., USA) using 5 mM H_2SO_4 solution as mobile phase at 0.6 mL/min, and the HPLC was operated at 65 °C. COD and ammonia-nitrogen in the fermentation broth was evaluated by Hach DR2700 Water Quality Analyzer (Hach Company, USA).

Biomass production was expressed as DCW (dry cell weight). The yeast cells were harvested from 5 mL of culture medium by centrifugation at 10,000 rpm for 10 min, washed twice with distilled water, dried to constant weight in an oven at 105 °C, and weighed.

Lipids were extracted in accordance with an adaptation (Bourque and Titorenko, 2009) of the method of Bligh and Dyer (Bligh and Dyer, 1959). Lipids were extracted from dry biomass with chloroform/methanol (2:1 v/v). The extracted lipids were centrifuged to obtain a clear supernatant and the solvent was removed by vacuum evaporation. Lipid yield was expressed as the amount of lipids extracted from the cells in per liter fermentation broth (g/L) and lipid content was defined as the percentage of lipid to dry biomass (%, w/w).

Polysaccharides were extracted from dry biomass through hydrothermal extraction method combined with repeated freeze-thaw method and ultrasonic treatment (Eom et al., 2022). And the content of polysaccharide in the extract was determined by phenol-sulfuric acid method (Zhou and Zhang, 2015), while glucose was used for the standards. Polysaccharide yield was expressed as the amount of cell-wall polysaccharides extracted from the cells per liter fermentation broth (g/L) and polysaccharide content was defined as the percentage of cell-wall polysaccharides to dry biomass (%, w/w).

3. Results and discussion

3.1. Consumption of carbohydrates by different oleaginous yeast during the single strain fermentation on EUOH

As demonstrated in Table 1, the sugars, contained in large amount in EUOH were the main carbon source in the fermentation broth, mainly including xylose (10.36 ± 1.06 g/L), glucose (9.62 ± 1.51 g/L), arabinose (3.27 ± 0.23 g/L) and cellobiose (1.31 ± 0.10 g/L). During the whole fermentation course, samples were taken regularly to detect the changes in the concentration of various sugars in the fermentation broth, the results are shown in Fig. 1. It could be seen that different strains showed different metabolic characteristics and consumption patterns of these carbohydrates.

Glucose was one of the two most abundant sugars in EUOH, and its content was only slightly lower than that of xylose. Compared with the other types of sugar, all the strains showed the fastest absorption and utilization of glucose as it was considered as a preferred carbon source for microorganism (Gao et al., 2022; Patel and Matsakas, 2019). As illustrated in Fig. 1A, within 48 h after inoculation, all the strains but *Y. lipolytica* consumed > 90% of the glucose, while this latter strain reached 81% consumption only. During the subsequent fermentation period (48–120 h), the decrease of glucose content was very slow and slight. At the end of fermentation, the final consumption rates of glucose in each culture were: *C. albidus* (97.7%), *L. Starkeyi* (97.5%), *R. glutinis* (95.7%), *R. toruloides* (95.0%), *T. cutaneum* (97.5%), *T. dermatis* (97.6%) and *Y. lipolytica* (86.0%) respectively, as shown in Table 2. It can be concluded that there was no significant difference in the consumption rate of glucose among all the strains except *Y. lipolytica*.

The content of xylose was the highest in EUOH. However, due to the carbon catabolite repression (CCR) of glucose over xylose (Zhao et al., 2020), the metabolism of xylose by yeasts lagged behind to a certain extent, and significant consumption began 24 h after inoculation (Fig. 1B). Moreover, different strains showed different xylose metabolic capacity. Among all the strains, *T. dermatis* and *T. cutaneum* showed a

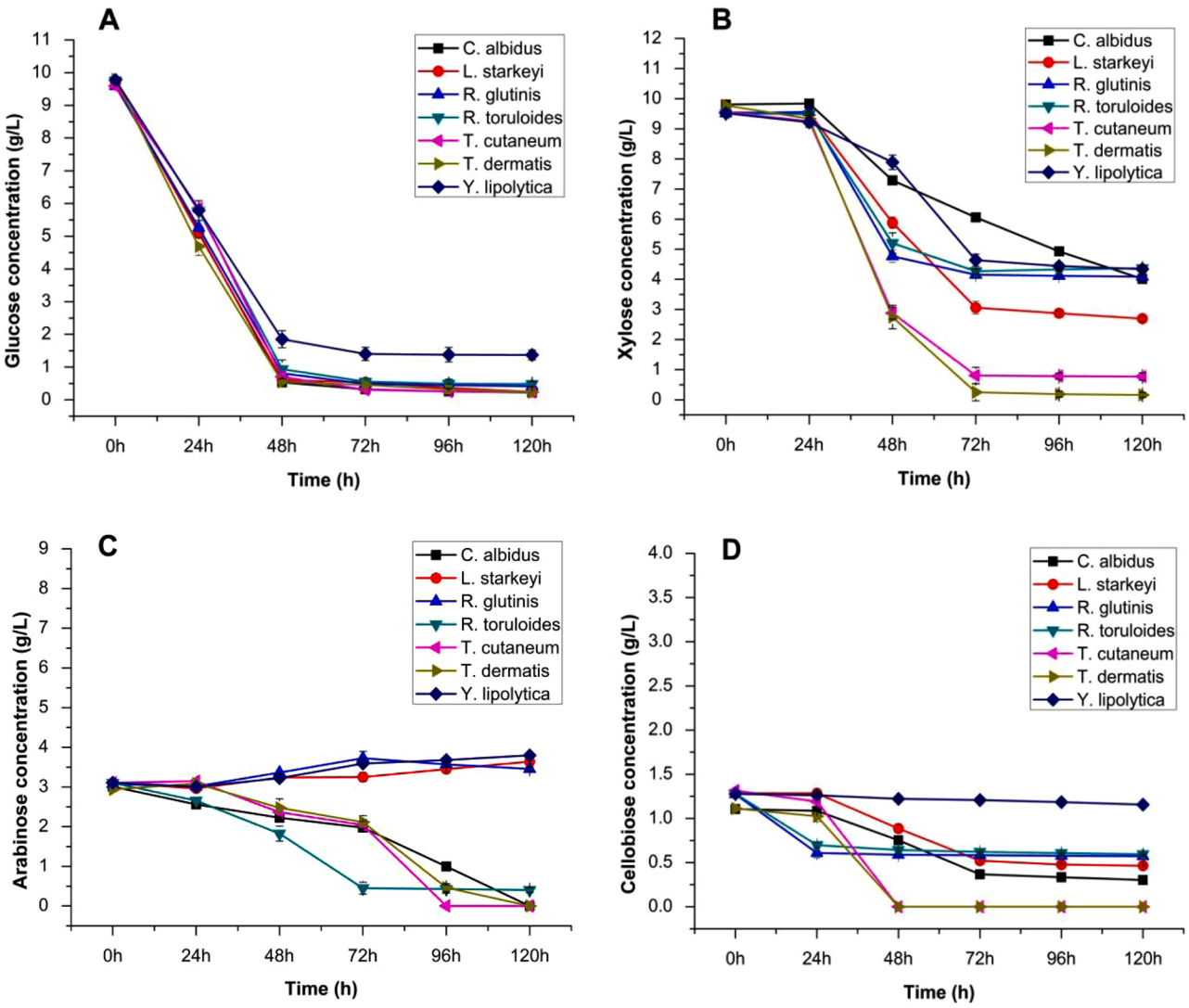

Fig. 1. Time profiles of the concentration of (A) glucose; (B) xylose; (C) arabinose; (D) cellobiose by different oleaginous yeasts for the single strain fermentation on EUOH.

quite good metabolic capacity for xylose and could consume almost all the xylose within 72 h after inoculation. At the end of fermentation, the consumption rates of xylose by *T. dermatis* and *T. cutaneum* were 98.3% and 91.9% respectively, which were equivalent to that of glucose (Table 2). The other strains showed much poorer capacity to metabolize xylose. The final consumption rates of xylose were *C. albidus* (59.1%), *L. Starkeyi* (71.8%), *R. glutinis* (57.0%), *R. toruloides* (54.0%) and *Y. lipolytica* (54.4%) respectively, which were much lower than that of glucose (Table 2).

The content of arabinose in EUOH was about 3–4 g/L. As another type of five-carbon sugar, the metabolic capacity of arabinose varied greatly among different strains (Fig. 1C). Specifically, *L. Starkeyi*, *R. glutinis* and *Y. lipolytica* could not metabolize arabinose at all during the cultivation period, and the detected concentration of arabinose at the end of fermentation was even slightly higher than the initial concentration, which might be the result of the continuous hydrolysis of a few incompletely hydrolyzed organic components in the fermentation broth. *C. albidus*, *R. toruloides, T. cutaneum* and *T. dermatis* showed a capacity to metabolize arabinose. Similar to xylose, the absorption and utilization of arabinose by these yeasts also lagged behind due to the CCR of glucose. Specifically, *C. albidus* and *R. toruloides* began to metabolize arabinose 24 h after inoculation while *T. cutaneum* and *T. dermatis* began to metabolize arabinose 48 h after inoculation. Among them, the strain of *R. toruloides* could rapidly metabolize most of arabinose within the period of 24–72 h, and then the absorption of arabinose slowed down, and its concentration was basically stable at 0.4–0.5 g/L until the end of fermentation, resulting in a arabinose consumption rate of 87.0% (Table 2). The strain of *C. albidus, T. cutaneum* and *T. dermatis* could metabolize arabinose gradually and completely within the 5-day cultivation period.

There was also a small amount (< 1.5 g/L) of cellobiose in EUOH. As shown in Fig. 1D, the metabolism of cellobiose by *R. toruloides* and *R. glutinis* did not lag significantly, almost synchronized with the metabolism of glucose, but the metabolic rate was low. Probably due to the low content of cellobiose in EUOH, about 50% of cellobiose could be consumed by *R. toruloides* and *R. glutinis* within 48 h after inoculation, but after that, the concentration of cellobiose was basically unchanged and stabilized at about 0.4–0.6 g / L. As shown in Table 2, at the end of fermentation, the consumption rate of cellobiose by *R. toruloides* and *R. glutinis* were quite close, which were 53.5% and 55.0%, respectively. The other strains could not metabolize cellobiose until 24 h after inoculation. Among them, *T. cutaneum* and *T. dermatis* showed a fast absorption for cellobiose and could reached a 100% consumption rate of cellobiose within the period of 24–48 h. The absorption of cellobiose by *C. albidus, L. Starkeyi* and *Y. lipolytica* was relatively slow and continued until the end of fermentation. The final consumption rate of cellobiose

Table 2
Sugars consumption rates of different oleaginous yeasts for the single strain and mixed strains fermentation on EUOH.

Batches and strains	Sugars consumption (%)[a]				Total sugars consumption (%)
	Glucose	Xylose	Arabinose	Cellobiose	
The single strain fermentation					
Cryptococcus albidus	97.7 ± 1.2	59.1 ± 1.7	100.0 ± 0.0	72.6 ± 0.9	80.7 ± 1.6
Lipomyces starkeyi *	97.5 ± 1.0	71.8 ± 0.6	0.0 ± 0.0	63.8 ± 1.2	70.2 ± 2.0
Rhodotorula glutinis	95.7 ± 0.7	57.0 ± 1.5	0.0 ± 0.0	55.0 ± 1.6	63.6 ± 2.4
Rhodosporidium toruloides *	95.0 ± 0.8	54.0 ± 1.8	87.0 ± 0.9	53.5 ± 1.5	75.3 ± 1.5
Trichosporon cutaneum *	97.5 ± 0.8	91.9 ± 2.0	100.0 ± 0.0	100.0 ± 0.0	95.7 ± 1.6
Trichosporon dermatis *	97.6 ± 1.1	98.3 ± 1.0	100.0 ± 0.0	100.0 ± 0.0	98.3 ± 1.0
Yarrowia lipolytica	86.0 ± 1.4	54.4 ± 2.2	0.0 ± 0.0	9.6 ± 0.8	55.0 ± 2.5
The mixed strains fermentation					
LS + RT	96.9 ± 0.9	67.8 ± 2.0	73.7 ± 1.7	65.8 ± 1.3	80.5 ± 1.2
LS + TC	98.4 ± 1.3	90.2 ± 0.9	94.4 ± 0.8	100.0 ± 0.0	94.5 ± 1.0
LS + TD	97.4 ± 1.0	98.9 ± 0.6	98.8 ± 0.8	100.0 ± 0.0	98.3 ± 0.7
RT + TC	97.3 ± 1.0	87.5 ± 1.4	92.2 ± 1.5	100.0 ± 0.0	92.6 ± 0.8
RT + TD	97.5 ± 1.0	98.4 ± 0.8	100.0 ± 0.0	100.0 ± 0.0	98.3 ± 0.5
TC + TD	98.1 ± 1.2	96.6 ± 1.0	100.0 ± 0.0	100.0 ± 0.0	97.9 ± 0.5

EUOH: the Eucommia ulmoides Oliver hydrolysate; LS: Lipomyces starkeyi; RT: Rhodosporidium toruloides; TC: Trichosporon cutaneum; TD: Trichosporon dermatis.
*The strains selected for the mixed strains fermentation.
[a] The sugar consumption (%) was calculated based on the residual sugar in the fermentation broth at the end of fermentation (120 h).

were *C. albidus* (72.6%), *L. Starkeyi* (63.8%) and *Y. lipolytica* (9.6%), respectively.

In addition to sugars, EUOH also contained 1–3 g/L of volatile fatty acids (VFAs), of which more than 70% was acetic acid, as well as a small amount of formic acid, propionic acid and butyric acid. In recent years, quite a few studies have reported that VFAs were available carbon sources for oleaginous yeast, and generally, the inhibition of VFAs of low concentration (< 5 g/L) on yeast cells could be ignored and oleaginous yeasts usually show a preference for acetic acid rather than other types of acids (Fei et al., 2011; Gao et al., 2020, 2017; Huang et al., 2018; Vajpeyi and Chandran, 2015). Consistent with previous studies, during the fermentation process in this study, all the strains showed the ability to metabolize VFAs. Especially for acetic acid, it was completely consumed in all cultures within 72 h after inoculation, leaving only trace amounts of formic, propionic and butyric acid remained at the end of fermentation (data not shown).

3.2. Growth and lipid production of different oleaginous yeast during the single strain fermentation on EUOH

The biomass and lipid production performance of different oleaginous yeasts cultured on EUOH are shown in Table 3. It is found that, at the end of fermentation, the biomass obtained by *T. cutaneum* and *T. dermatis* were 17.37 and 16.23 g/L, respectively, which were significantly more than that of the other strains. Actually, as illustrated in Fig. 2, the cells of *T. cutaneum* and *T. dermatis* proliferated rapidly within 48 h after inoculation (i.e. logarithmic growth period), and reached their maximum biomass within the period of 48–72 h, which were 25.80

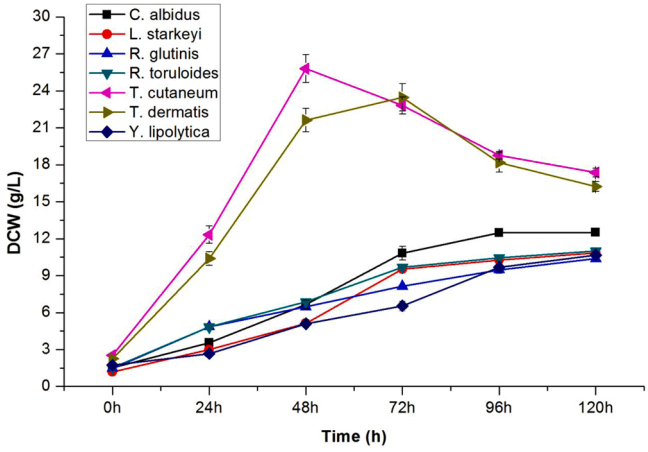

Fig. 2. Cell growth profiles of different oleaginous yeasts measured as dry cell weight (DCW) for the single strain fermentation on EUOH.

Table 3
Biomass, lipid and polysaccharide production of different oleaginous yeasts in single and mixed strains fermentation on EUOH after 120 h.

Batches and strains	Biomass (g/L)	Lipid content (wt%)	Lipid yield (g/L)	Polysaccharide content (wt%)	Polysaccharide yield (g/L)
The single strain fermentation					
Cryptococcus albidus	12.50 ± 0.30	18.76	2.35 ± 0.05	—[a]	—
Lipomyces starkeyi *	10.83 ± 0.25	34.74	3.76 ± 0.08	9.36	1.01 ± 0.02
Rhodotorula glutinis	10.38 ± 0.22	25.37	2.63 ± 0.04	—	—
Rhodosporidium toruloides *	11.00 ± 0.32	30.15	3.32 ± 0.08	16.12	1.77 ± 0.05
Trichosporon cutaneum *	17.37 ± 0.50	13.45	2.34 ± 0.03	7.91	1.37 ± 0.04
Trichosporon dermatis *	16.23 ± 0.45	12.16	1.97 ± 0.05	6.14	1.00 ± 0.02
Yarrowia lipolytica	10.67 ± 0.28	9.85	1.05 ± 0.03	—	—
The mixed strains fermentation					
LS + RT	12.09 ± 0.23 (**A**)	31.57 (**B**)	3.82 ± 0.06 (**A**)	13.53 (**B**)	1.64 ± 0.03 (**B**)
LS + TC	19.75 ± 0.35 (**A**)	15.84 (**B**)	3.13 ± 0.05 (**B**)	8.19 (**B**)	1.62 ± 0.03 (**A**)
LS + TD	19.93 ± 0.40 (**A**)	13.95 (**B**)	2.78 ± 0.05 (**B**)	7.56 (**B**)	1.51 ± 0.03 (**A**)
RT + TC	19.57 ± 0.37 (**A**)	15.77 (**B**)	3.09 ± 0.05 (**B**)	11.89 (**B**)	2.33 ± 0.04 (**A**)
RT + TD	19.50 ± 0.30 (**A**)	13.05 (**B**)	2.54 ± 0.03 (**B**)	12.19 (**B**)	2.38 ± 0.04 (**A**)
TC + TD	21.57 ± 0.52 (**A**)	13.02 (**B**)	2.81 ± 0.04 (**A**)	8.68 (**A**)	1.87 ± 0.03 (**A**)

EUOH: the Eucommia ulmoides Oliver hydrolysate; LS: Lipomyces starkeyi; RT: Rhodosporidium toruloides; TC: Trichosporon cutaneum; TD: Trichosporon dermatis. (**A**): The determination result was higher than both of the corresponding two single strain fermentations; (**B**): The determination result was in between the values observed for the two corresponding single strain fermentations.
* The strains selected for the mixed strains fermentation.
[a] The polysaccharide content of unselected strains was not determined.

and 23.47 g/L, respectively. After that, the biomass of these two strains decreased obviously, and basically remained unchanged after the 72 h. Such significant biomass decline followed after a rapid cell proliferation stage had also occurred when we were studying some other strains of high vitality in batch cultures (Gao et al., 2022). We mainly attribute this phenomenon to the strong reproductive activity of these strains and the limited nutrients in batch cultivation mode, which leads to the insufficiency of nutrients in the latter stage of culture, so that yeast cells entered a starvation state, stopped growing and began to decay. Except *T. cutaneum* and *T. dermatis,* the biomass of all the other strains increased continuously during the whole cultivation duration, and after 72 h of inoculation, the cell growth rate slowed down and the biomass gradually stabilized (Fig. 2). At the end of fermentation, the biomass obtained by *C. albidus, L. Starkeyi, R. glutinis, R. toruloides* and *Y. lipolytica* were 12.50, 10.83, 10.38, 11.00 and 10.67 g/L, respectively.

Lipid production depends not only on yeast biomass, but also on the lipid content in yeast cells. It is found that, compared with the other strains, *L. Starkeyi* and *R. toruloides* could synthesize significantly more lipids in cells. As shown in Table 3, at the end of fermentation, the lipid contents obtained by *L. Starkeyi* and *R. toruloides* were 34.74% and 30.15% respectively, followed by *R. glutinis* with a lipid content of 25.37%. The lipid content of the other strains was much lower, all less than 20%, which were *C. albidus* (18.76%), *T. cutaneum* (13.45%), *T. dermatis* (12.16%) and *Y. lipolytica* (9.85%), respectively. In summary, when using EUOH as the fermentation substrate, the highest lipid yield was obtained by *L. Starkeyi*, which was 3.76 g/L, and followed by *R. toruloides*, which was 3.32 g/L. Although the cells of *T. cutaneum* and *T. dermatis* grew vigorously and could accumulate a large amount of biomass, their lipid yield was only 2.34 g/L and 1.97 g/L due to their low intracellular lipid content. And the lipid yield of *C. albidus, R. glutinis* and *Y. lipolytica* were 2.35, 2.63 and 1.05 g/L, respectively. It is worth mentioning that lipid-producing fermentation on sugars or other hydrophilic substrates (de novo lipid accumulation) usually requires limited nitrogen sources and relatively high C/N ratios (Gao et al., 2022; Zheng et al., 2012; Papanikolaou and Aggelis, 2011; Zhan et al., 2016). The favorable range of C/N ratio for de novo lipid accumulation was reported to be 30–80 (Moreton, 1988). In the present study, the initial C/N ratio of all the cultures on EUOH (after inoculation) was 5.2–6.6, far below the favorable range. It indicates that the nitrogen content in EUOH was too high for the lipid accumulation in oleaginous yeast cells. Thus, by means of adjusting the C/N ratio of fermentation broth, the lipid content and lipid production could be further improved. It could be studied in the future research.

3.3. Investigation on the function and effect of the mixed strains fermentation (compared with the single strain fermentation)

Mixed strains fermentation has been considered to be one of the effective means to optimize the overall fermentation performance on substrates with complex components or containing inhibitors, as it could provide a more stable, active and diverse yeast cell community with comprehensive metabolic characteristics (Finneran and Popovic, 2018; Qi et al., 2015; Schlembach et al., 2021). Based on the above research results, *T. cutaneum* and *T. dermatis*, which could metabolize all types of sugars in EUOH quite well and accumulate a large amount of biomass, as well as *L. starkeyi* and *R. toruloides*, which could synthesize a high intracellular lipid content, were selected for the further research on the mixed strains fermentation. Compared with the single strain fermentation, the function and effect of the mixed strains fermentation were systematically investigated and analyzed in terms of substrate utilization, lipid and polysaccharide production as well as COD and ammonia-nitrogen removals. The results and analysis are as follows.

3.3.1. Consumption of carbohydrates

After 5 days of fermentation with the selected strains, the contents of various residual sugars in the fermentation broth were analyzed, and the corresponding consumption rates were calculated. As shown in Table 2, in the single strain fermentation of *L. starkeyi,* arabinose could not be absorbed and utilized. But when *L. starkeyi* was mixed-cultured with any of *R. toruloides, T. cutaneum* and *T. dermatis,* an arabinose consumption rate of 73.7% (LS+RT), 94.4% (LS+TC) and 98.9% (LS+TD) had been achieved respectively. Besides, as *L. starkeyi* and *R. toruloides* could not metabolize xylose and cellobiose very well, the xylose consumption rates in their single strain fermentation were 71.8% and 54.0% respectively, and the cellobiose consumption rates were 63.8% and 53.5% respectively. By mixed culture with *T. cutaneum* or *T. dermatis,* the consumption rate of xylose could increased to 92.2% (LS+TC), 98.9% (LS+TD), 87.5% (RT+TC) and 98.4% (RT+TC), respectively, and the consumption rate of cellobiose had all achieved 100%. It could be concluded that, compared with single strain fermentation, mixed strains fermentation could effectively give full play to the metabolic advantages of different strains on various substrates, making various carbon source substances in fermentation substrates more fully absorbed and utilized.

Therefore, it could be inferred that a high consumption rate of total sugars could be obtained through mixed-culture with strains that have high consumption rates of various sugars, which had been confirmed in this study. As shown in Table 2, the total sugar consumption rate of all the mixed strains fermentation with *T. cutaneum* or *T. dermatis,* the strains that could metabolize all types of sugars quite well, had exceeded 90%. In addition, despite the relatively low sugar utilization efficiency of *L. starkeyi* and *R. toruloides*, the total sugar consumption rate of the mixed fermentation (LS+RT), which was 80.5%, was much higher than that of the single strain fermentation of *L. starkeyi* (70.2%) and *R. toruloides* (75.3%). The results indicated that the fermentation efficiency of the mixed cultures was superior to that of the corresponding single strain fermentation. It was probably because mixed strains fermentation could create a competitive state between different strains which could further stimulates the active growth and metabolism of strains, and similar conclusions had also been reported by Finneran and Popovic (2018) when studied the Co- and mixed cultures for xylose fermentation.

3.3.2. Growth and lipid production

The biomass and lipid production of the mixed strains fermentations on EUOH were demonstrated in Table 3. It could be seen that the mixed strains fermentation could effectively promote the cell growth, making the total biomass significantly greater than that of the corresponding single strain fermentation. In particular, when the two vigorous growing strains, i.e. *T. cutaneum* and *T. dermatis*, were mixed-cultured, the maximum biomass of 21.57 g/L could be obtained. The biomass production of the mixed strains fermentation (LS+RT), (LS+TC), (LS+TD), (RT+TC), (RT+TD) had reached 12.09, 19.75, 19.93, 19.57, 19.50 g/L, respectively. It could also be seen from Table 3 that the overall lipid content obtained by the mixed strains fermentation was between those of the corresponding two single strain fermentations, and was closer to the lipid content of the strain with vigorous growth. This result seems to indicate that the mixed strains fermentation could not stimulate the yeast cells to synthesize more lipid, and the overall lipid content measured was the result of the mixing of cells with different lipid content, which was related to the proportion of cells in different strains.

On the whole, although mixed culture of two different strains could greatly increase the biomass, the lipid yield had no significant improvement due to the “compromise” of the overall lipid content. In this study, the lipid yield of the mixed strains fermentation was usually between the lipid yields obtained by the corresponding two single strain fermentations, more specifically, it was much higher than that of the one with lower lipid yield, but was usually slightly lower than that of the other one with higher lipid yield. Additionally, it should be noted that there had been a certain increase in lipid yield of the mixed cultures of two different strains with similar lipid content and biomass, i.e., the fermentation of (LS+RT) and (TC+TD). As shown in Table 3, the lipid yield of the single strain fermentations of *L. starkeyi, R. toruloides, T.*

cutaneum and *T. dermatis* were 3.76, 3.32, 2.34 and 1.97 g/L, respectively, while the lipid yield of the mixed strains fermentation (LS+RT), (TC+TD) had increased to 3.82 g/L and 2.81 g/L, respectively.

3.3.3. Removal of COD and ammonia-nitrogen

In order to comprehensively analyze and compare the mixed strains fermentation with single strain fermentation, COD and ammonia-nitrogen removals were measured and evaluated. The results are shown in Table 4. Among the single strain fermentations, due to the higher sugar consumption rate and stronger cell growth activity, both of the COD and ammonia-nitrogen removal rates of *T. cutaneum* and *T. dermatis* were higher than those of *L. starkeyi* and *R. toruloides*. Specifically, the ammonia-nitrogen removal rates of *T. cutaneum* and *T. dermatis* were 81.8% and 80.4% respectively, while those of *L. starkeyi* and *R. toruloides* were 73.0% and 71.8% respectively. The COD removal rates of *T. cutaneum* and *T. dermatis* were 66.4% and 65.4% respectively, while those of *L. starkeyi* and *R. toruloides* were 59.8% and 54.2% respectively.

It is found that the ammonia-nitrogen removal rates of the mixed strains fermentation (LS+RT), (LS+TC), (LS+TD), (RT+TC), (RT+TD) and (TD+TD) were 74.9%, 79.6%, 81.0%, 81.4%, 80.4% and 84.2% respectively, which were not much different from that of the corresponding single strain fermentation (the one with better ammonia-nitrogen removal effect). The results indicated that mixed strains fermentation could not significantly improve the ammonia-nitrogen removal rate. The possible reason is that, as shown in Table 2, carbon sources were depleted before nitrogen sources. The lack of carbon sources would restrict the absorption and utilization of nitrogen nutrition in the latter stage of fermentation, resulting in no significant improvement in ammonia-nitrogen removal rate in the mixed strains fermentation. Different from the ammonia-nitrogen removal rate, the COD removal rate in the mixed strains fermentation had been significantly improved. As shown in Table 4, the COD removal rates of the mixed strains fermentation (LS+RT), (LS+TC), (LS+TD), (RT+TC), (RT+TD) and (TD+TD) were 67.4%, 75.2%, 76.6%, 77.7%, 74.9% and 79.5% respectively, which were obviously higher than those of the corresponding single strain fermentations. The improvement of COD removal rate was mainly attributed to the substantial increase of overall biomass in the mixed strains fermentation. In addition, the more active cellular metabolic activity stimulated by the competitive state in mixed culture could also help to improve the COD removal rate.

3.3.4. Yeast polysaccharide production

Based on the above research and analysis, it could be concluded that the mixed strains fermentation could obtained a high consumption rate of total sugars, greatly improve the COD removal rate and significantly increase the total biomass, but it had no obvious effect on improving the lipid production. These results indicated that in the mixed strains fermentation, a large number of carbohydrates absorbed and metabolized by these oleaginous yeasts were not transformed into intracellular lipids, but were used to synthesize other cell components. Polysaccharides are the major components of the yeast cell wall and play multiple functions, ranging from the carriers of immunochemical specificity and marker molecules, by which cells recognize each other and interact with the environment, to the skeletal substances that define stability, shape, and morphology of the cell (Cheng et al.,2012; Kogan et al., 2008). Due to their novel physiochemical characteristics and compositions, microbial polysaccharides have gained high importance and broad-scale applications in biotechnology (Qamar et al., 2022). Therefore, we had determined and analyzed the polysaccharide content and polysaccharide yield of the dry biomass obtained from each cultures, and the results are shown in Table 3 as well.

In the single strain fermentation of the four selected strains on EUOH, the cells of *R. toruloides* had the highest polysaccharide content of 16.12%, and its polysaccharide yield was also the highest (1.77 g/l). The polysaccharide content and yield of the other strains were much lower. Specifically, *L. starkeyi*, *T. cutaneum* and *T. dermatis* had a polysaccharide content of 9.36, 7.91, 6.14% respectively, and obtained a polysaccharide yield of 1.01, 1.37, 1.00 g/L respectively. It is found that except the fermentation (LS+RT), all the mixed strains fermentations had obtained a much higher polysaccharide yield than that of the corresponding two single strain fermentations. As shown in Table 3, the yeast polysaccharide yield of the fermentation (LS+TC), (LS+TD), (RT+TC), (RT+TD) and (TC+TD) reached 1.62, 1.51, 2.33, 2.38 and 1.87 g/L respectively, which increased by 17.73%, 48.64%, 31.22%, 34.05% and 36.27% respectively compared with the corresponding single strain fermentation with higher polysaccharide yield, and even increased by 59.6%, 51.2%, 69.4%, 138.5% and 87.9% respectively compared with the corresponding single strain fermentation with lower polysaccharide yield. For the fermentation (LS+RT), the polysaccharide yield (1.64 g/L) was much higher than that of *L. starkeyi* (1.01 g/L), but slightly lower than that of *R. toruloides* (1.77 g/L).

The yield of polysaccharide was mainly related to biomass and cell polysaccharide content. In this present study, the substantial increase of polysaccharide yield was mainly attributed to the substantial increase of the overall biomass in the mixed strains fermentation. As for the fermentation (LS+RT), the increase of biomass was not obvious due to the relatively weak growth activity of both *L. starkeyi* and *R. toruloides*, so the polysaccharide yield did not exceed that of the single strain fermentation of *R. toruloides*. In terms of polysaccharide content, due to the mixing of two yeasts with different polysaccharide content, the measured overall polysaccharide content in the mixed strains fermentation was usually between the two polysaccharide content values of the corresponding two single strain fermentations. However, different from the measured overall lipid content of mixed culture, which was naturally closer to that of the strain with strong growth activity (e.g. *T. cutaneum*

Table 4
COD and ammonia-nitrogen removals in single and mixed strain fermentations on EUOH by oleaginous yeasts after 120 h.

Batches and strains	COD				Ammonia-nitrogen			
	Initial value (mg/L)	Final value (mg/L)	Consumption (mg/L)	Removal (%)	Initial value (mg/L)	Final value (mg/L)	Consumption (mg/L)	Removal (%)
The single strain fermentation								
L. starkeyi	51620	20770	30850	**59.8**	1809.6	488.4	1321.2	**73.0**
R. toruloides	51920	23780	28140	**54.2**	1754.8	495.0	1259.8	**71.8**
T. cutaneum	51470	17310	34160	**66.4**	1826.4	331.6	1494.8	**81.8**
T. dermatis	49860	17270	32590	**65.4**	1902.0	372.0	1530.0	**80.4**
The mixed strains fermentation								
LS + RT	50870	16590	34280	**67.4**	1786.6	449.2	1337.4	**74.9**
LS + TC	52970	13120	39850	**75.2**	1922.4	392.4	1530.0	**79.6**
LS + TD	53120	12420	40700	**76.6**	1965.6	374.4	1591.2	**81.0**
RT + TC	52820	11770	41050	**77.7**	1794.2	333.8	1460.4	**81.4**
RT + TD	51770	13020	38750	**74.9**	1764.4	346.0	1418.4	**80.4**
TC + TD	52220	10690	41530	**79.5**	1942.8	306.8	1636.0	**84.2**

EUOH: the Eucommia ulmoides Oliver hydrolysate; LS: Lipomyces starkeyi; RT: Rhodosporidium toruloides; TC: Trichosporon cutaneum; TD: Trichosporon dermatis.

and *T. dermatis*), the polysaccharide content measured in these mixed strains fermentations had no such trend. Compared with the low polysaccharide content of *T. cutaneum* and *T. dermatis* with the dominant cell number in the mixed culture, the overall polysaccharide content of the mixed strains fermentations had been greatly improved, not just an average result after mixing. Especially for the fermentation (TC+TD), its polysaccharide content (8.68%) was even higher than that of the corresponding two single strain fermentations, i.e. 7.91% (*T. cutaneum)* and 6.14% (*T. dermatis*). These results indicated that mixed strains fermentation could promote the oleaginous yeast to synthesize more polysaccharides. It could be speculated that, stimulated by the competitive environment in mixed culture, the structure of yeast cell wall would change, which could promote the increase of polysaccharide content in the cell structure to strengthen cell wall stability and cell morphology (Giovani et al., 2009), and this change could be more obvious in the mixed culture of strains with strong growth activity, e.g. the fermentation (TC+TD).

3.4. Evaluation on the impacts of the mixed strains fermentation

Based on the above research results, an evaluation of the impacts of the mixed strains fermentation, compared with the corresponding single strain fermentation, was summarized in Table 5. It is found that the mixed strains fermentation could effectively promote a more comprehensive and thorough utilization of fermentation substrates and stimulate a more active growth activity of yeast cells, making the effects of substrates consumption, biomass accumulation and COD removal better than both of the two corresponding single strain fermentations. In terms of the ammonia-nitrogen removal effect, probably due to the lack of carbon sources in the latter stage of fermentation, which restrict the absorption and utilization of nitrogen nutrition, the mixed strains fermentation was only found to be comparable to that of the corresponding single strain fermentation with strong ammonia-nitrogen removal effect, not greatly improved. Both of the lipid yield and polysaccharide yield are closely related to the transformation of carbon source nutrients absorbed and metabolized by yeast in cells. As shown Table 5, the lipid yield of the mixed strains fermentation was significantly improved when compared with the corresponding single strain fermentation with low lipid yield; However, due to the "compromise" of the overall lipid content (detailed in Section 3.3.3), the lipid yield of the mixed strains fermentation was usually inferior to that of the corresponding single strain fermentation with high lipid yield, or only slightly improved. Different from the lipid yield, the polysaccharide yield of the mixed strains fermentation was found to be much higher than both of the two corresponding single strain fermentations. Nevertheless, for the case that the lipid yield of the mixed strains fermentation was better than both of the two corresponding single strain fermentations, its polysaccharide yield could be slightly lower than that of the corresponding single strain fermentation with large polysaccharide yield.

In this study, among all the mixed strains fermentation, when the two strains with the highest lipid content (i.e. *L. starkeyi* and *R. toruloides*) were mixed-cultured, the maximum lipid yield of 3.82 g/L was achieved, and the yeast polysaccharide yield, COD and ammonia-nitrogen removal rates of the fermentation (LS+RT) were 1.64 g/L, 67.4% and 74.9% respectively. When the strain with the highest polysaccharide content (i.e. *R. toruloides*) was mixed-cultured with the strains with strong growth activity (i.e. *T. cutaneum* and *T. dermatis*), a large amount of yeast polysaccharides could be obtained, which were 2.33 g/L (RT+TC) and 2.38 g/L (RT+TD) respectively. And the lipid yield, COD and ammonia-nitrogen removal rates of the fermentation (RT+TC), (RT+TD) were 3.09 g/L, 77.7%, 81.4% and 2.54 g/L, 74.9%, 80.4%, respectively.

Table 5
Evaluation on the impacts of the mixed strains fermentation (compared with the single strain fermentation).

Item	Evaluation on the impacts of the mixed strains fermentation
Substrates consumption	Better than both of the two corresponding single strain fermentations
Biomass	Better than both of the two corresponding single strain fermentations
Lipid yield	Significantly improved when compared with the corresponding single strain fermentation with low lipid yield; However, usually inferior to the other single strain fermentation with high lipid yield, or only slightly improved
Polysaccharide yield	Usually* better than both of the two corresponding single strain fermentations
COD removal	Better than both of the two corresponding single strain fermentations
Ammonia-nitrogen removal	Comparable to that of the corresponding single strain fermentation with strong ammonia-nitrogen removal effect

*For the case that the lipid yield of the mixed strains fermentation was better than both of the two corresponding single strain fermentations, its polysaccharide yield could be slightly lower than that of the corresponding single strain fermentation with large polysaccharide yield.

4. Conclusions

Eucommia ulmoides Oliver hydrolysate (EUOH), which contains abundant and diverse sugars including xylose, glucose, arabinose and cellobiose.etc, could be well fermented by oleaginous yeasts. Compared with single strain fermentation, it was found that the mixed strains fermentation could effectively promote a more comprehensive and thorough utilization of the various sugars in EUOH, greatly improve biomass and yeast polysaccharide production, as well as COD removal effect, but could not significantly improve the overall lipid content and ammonia nitrogen removal effect. Still, due to the substantial increase of biomass, the lipid production could be improved to a certain extent.

CRediT authorship contribution statement

Ruiling Gao: Conceptualization, Methodology, Validation, Investigation, Writing – original draft. **Hairong Zhang:** Methodology, Resources, Writing – review & editing. **Lian Xiong:** Resources, Writing – review & editing. **Hailong Li:** Investigation, Writing – review & editing. **Xuefang Chen:** Methodology, Writing – review & editing. **Mengkun Wang:** Validation, Investigation. **Xinde Chen:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

The authors acknowledge the financial support of the Henan Province-CAS Scientific and Technical Payoffs Transformation Project (2021114), the Guangzhou Science and Technology Planning Project (202201010229), the Project of Jiangsu Province Science and Technology (BE2020394), the project of Guangdong Provincial Key Laboratory of New and Renewable Energy Research and Development (E1390104), and the project of National Natural Science Foundation of China (51876207, 52006229).

References

Besson, M., Gallezot, P., Pinel, C., 2014. Conversion of biomass into chemicals over metal catalysts. Chem. Rev. 114 (3), 1827–1870.

Bligh, E., Dyer, W.J., 1959. A rapid method of total lipid extraction and purifcation. Can. J. Biochem Physiol. 37, 911–917.

Bourque, S.D., Titorenko, V.I., 2009. A quantitative assessment of the yeast lipidome using electrospray ionization mass spectrometry. J. Vis. Exp. JoVE. https://doi.org/10.3791/1513.

Broos, W., Wittner, N., Geerts, J., Dries, J., Vlaeminck, S.E., Richel, A., Cornet, I., 2022. Evaluation of lignocellulosic wastewater valorization with the oleaginous yeasts *R. kratochvilovae* EXF7516 and *C. oleaginosum* ATCC 20509. Fermentation 8 (5), 204.

Cai, Z., Zhang, B., Li, Y., 2012. Engineering *Saccharomyces cerevisiae* for efficient anaerobic xylose fermentation: Reflections and perspectives. Biotechnol. J. 7, 34–46.

Cheirsilp, B., Suwannarat, W., Niyomdecha, R., 2011. Mixed culture of oleaginous yeast Rhodotorula glutinis and microalga *Chlorella vulgaris* for lipid production from industrial wastes and its use as biodiesel feedstock. N. Biotechnol. 28 (4), 362–368.

Chen, X.F., Huang, C., Xiong, L., Chen, X.D., Chen, Y., Ma, L.L., 2012. Oil production on wastewaters after butanol fermentation by oleaginous yeast *Trichosporon coremiiforme*. Bioresour. Technol. 118, 594–597.

Cheng, J.G., Zhang, S.F., Zhang, L., Zhao, C.X., Guo, J.Q., 2012. Analysis of molecular weight distribution and primary structural of yeast cell wall polysaccharides. Sci. Technol. Food Ind. 33 (12), 143–146.

Cho, H.U., Park, J.M., 2018. Biodiesel production by various oleaginous microorganisms from organic wastes. Bioresour. Technol. 256, 502–508.

Cianchetta, S., Polidori, N., Vasmara, C., Ceotto, E., Marchetti, R., Galletti, S., 2022. Single cell oil production from hydrolysates of alkali pre-treated giant reed: High biomass-to-lipid yields with selected yeasts. Ind. Crops Prod. 178 (2022), 114596.

Dias, C., Santos, J., Reis, A., Silva, T.L., 2019. Yeast and microalgal symbiotic cultures using low-cost substrates for lipid production. Bioresour. Technol. Rep. 7, 100261.

Eom, S.J., Park, J.T., Kang, M.C., Lee, N.H., Song, K.M., 2022. Use of ultrasound treatment to extract mannan polysaccharide from Saccharomyces cerevisiae. J. Food Process Eng. 2022, e14105.

Fei, Q., Chang, H.N., Shang, L., Choi, J.D., Kim, N., Kang, J., 2011. The effect of volatile fatty acids as a sole carbon source on lipid accumulation by *Cryptococcus albidus* for biodiesel production. Biores Technol. 102, 2695–2701.

Finneran, K.T., Popovic, J., 2018. Solvent production from xylose. Appl. Microbiol. Biotechnol. 102, 8707–8715.

Gao, R.L., Li, Z.F., Zhou, X.Q., Cheng, S.K., Zheng, L., 2017. Oleaginous yeast *Yarrowia lipolytica* culture with synthetic and food waste-derived volatile fatty acids for lipid production. Biotechnol. Biofuels 10, 247–262.

Gao, R.L., Li, Z.F., Zhou, X.Q., Bao, W.J., Cheng, S.K., Zheng, L., 2020. Enhanced lipid production by *Yarrowia lipolytica* cultured with synthetic and waste-derived high-content volatile fatty acids under alkaline conditions. Biotechnol. Biofuels 13, 3–19.

Gao, R.L., Xiong, L., Wang, M.K., Peng, F., Zhang, H.R., Chen, X.D., 2022. Production of acetone-butanol-ethanol and lipids from sugarcane molasses via coupled fermentation by *Clostridium acetobutylicum* and oleaginous yeasts. Ind. Crops Prod. 185 (2022), 115131.

Ghindea, R., Csutak, O., Stoica, I., Tanase, A.M., Vassu, T., 2010. Production of xylitol by yeasts. Rom. Biotechnol. Lett. 15 (3), 5217–5222.

Giovani, G., Canuti, V., Rosi, I., 2009. Effect of yeast strain and fermentation conditions on the release of cell wall polysaccharides. Int. J. Food Microbiol. 137, 303–307.

Huang, C., Guo, H.J., Zhang, H.R., Xiong, L., Li, H.L., Chen, X.D., 2019. A new concept for total components conversion of lignocellulosic biomass: a promising direction for clean and sustainable production in its bio-refinery. J. Chem. Technol. Biotechnol. 94 (8), 2416–2424.

Huang, X.F., Shen, Y., Luo, H.J., Liu, J.N., Liu, J., 2018. Enhancement of extracellular lipid production by oleaginous yeast through preculture and sequencing batch culture strategy with acetic acid. Bioresour. Technol. 247, 395–401.

Kamal, R., Huang, Q.T., Wang, Q., Yu, X., Song, J.Y., Limtong, S., Zhao, Z.B.K., 2022. Co-utilization of amino acid-rich wastes and glycerol for microbial lipid production. Biofuels Bioprod. Bioref. 16, 142–154.

Kang, H.L., Yao, L., Li, Y.S., Hu, X.R., Yang, F., Fang, Q.H., Zhang, L.Q., 2018. Highly toughened polylactide by renewable *Eucommia ulmoides* gum. J. Appl. Polym. Sci. 135, 12.

Kogan, G., Kocher, A., 2007. Role of yeast cell wall polysaccharides in pig nutrition and health protection. Livest. Sci. 109, 161–165.

Kogan, G., Pajtinka, M., Babincova, M., Miadokova, E., Rauko, P., Slamenova, D., Korolenko, T.A., 2008. Yeast cell wall polysaccharides as antioxidants and antimutagens: can they fight cancer? Minireview. NEOPLASMA 55 (5), 387–393.

Li, Q., Kamal, R., Wang, Q., Yu, X., Zhao, Z.K., 2020. Lipid Production from amino acid wastes by the oleaginous yeast *Rhodosporidium toruloides*. Energies 13 (07), 1576.

Li, Z.H., Wu, G.P., Liu, L., Zhao, M.M., Fan, X., Geng, T.Y., Gong, D.Q., 2022. Effects of yeast polysaccharide on performance, immune function, serum biochemical indices and antioxidant capacity of laying hens during late laying period. Chin. J. Anim. Nutr. 34 (4), 2413–2424.

Liu, G.H., Zhang, Y.K., Xiao, M.F., Peng, X.J., 2010. Study on extracting Eucommia ulmoides oliv. gum by cellulase pretreatment. Chem. Ind. For. Prod. 30 (02), 77–82.

Papanikolaou, S., Aggelis, G., 2011. Lipids of oleaginous yeasts. Part II: technology and potential applications. Eur. J. Lipid Sci. Technol. 113 (8), 1052–1073.

Patel, A., Matsakas, L., 2019. A comparative study on *de novo* and *ex novo* lipid fermentation by oleaginous yeast using glucose and sonicated waste cooking oil. Ultrason. - Sonochem. 52, 364–374.

Pereira, A.S., Lopes, M., Miranda, S.M., Belo, I., 2022. Bio-oil production for biodiesel industry by *Yarrowia lipolytica* from volatile fatty acids in two-stage batch culture. Appl. Microbiol. Biotechnol. 106 (8), 2869–2881.

Qamar, S.A., Riasat, A., Jahangeer, M., Fatima, R., Bilal, M., Iqbal, H.M.N., Mu, B.Z., 2022. Prospects of microbial polysaccharides-based hybrid constructs for biomimicking applications. J. Basic Microbiol. 2021, 1–18.

Qi, G.X., Xiong, L., Huang, C., Chen, X.F., Lin, X.Q., Chen, X.D., 2015. Solvents production from a mixture of glucose and xylose by mixed fermentation of *Clostridium acetobutylicum* and Saccharomyces cerevisiae. Appl. Biochem Biotechnol. 177 (4), 996–1002.

Qin, L., Liu, L., Zeng, A.P., Wei, D., 2017. From low-cost substrates to single cell oils synthesized by oleaginous yeasts. Bioresour. Technol. 245, 1507–1519.

Qin, L., Wang, J.B., Zhang, Y., Zhang, J.C., Fan, Y.M., 2021. Technical progress in extraction of natural polymer *Eucommia ulmoides* gum. Mod. Chem. Ind. 41 (12), 53–58, 63.

Rodrigues, G., Pais, C., 2000. The infuence of acetic and other weak carboxylic acids on growth and cellular death of the yeast *Yarrowia lipolytica*. Food Technol. Biotechnol. 38 (1), 27–32.

Royce, L.A., Liu, P., Stebbins, M.J., Hanson, B.C., Jarboe, L.R., 2013. The damaging efects of short chain fatty acids on *Escherichia coli* membranes. Appl. Microbiol Biothchnol 97, 8317–8327.

Schlembach, I., Tehrani, H.H., Blank, L.M., Buchs, J., Wierckx, N., Regestein, L., Rosenbaum, M.A., 2020. Consolidated bioprocessing of cellulose to itaconic acid by a co-culture of *Trichoderma reesei* and *Ustilago maydis*. Biotechnol. Biofuels 13, 207.

Schlembach, I., Grünberger, A., Rosenbaum, M.A., Regestein, L., 2021. Measurement techniques to resolve and control population dynamics of mixed-culture processes. Trends Biotechnol. 39 (10), 1093–1109.

Sun, Q.Q., Zhao, X.K., Wang, D.M., Dong, J.N., She, D., Peng, P., 2018. Preparation and characterization of nanocrystalline cellulose/*Eucommia ulmoides* gum nanocomposite film. Carbohydr. Polym. 181, 825–832.

Suutari, M., Liukkonen, K., Laakso, S., 1990. Temperature adaption in yeast: the role of fatty acids. J. Gen. Microbiol 136, 1469–1474.

Urazgaliev, K.S., Mynkina, G.I., Pravotorov, G.V., Demidenko, N.Y., Safina, A.F., Kashkina, M.A., Korolenko, T.A., 1992. Effect of stimulation of mononuclear phagocyte system by yeast polysaccharides on endocytosis by liver cells. Bull. Exp. Biol. Med. 114 (9), 1295–1298.

Vajpeyi, S., Chandran, K., 2015. Microbial conversion of synthetic and food wastederived VFA to lipids. Bioresour. Technol. 188, 49–55.

Wang, H.X., Tang, Y.H., Gao, H.Y., Gao, H., Zhao, Q.Y., Li, J.W., Xin, F.X., Zhang, W.M., Jiang, M., 2022. Microbial co-culturing of *Chlorella sp.* and *Rhodotorula toruloides* for the enhanced microbial lipid production. Fuel 324, 124609.

Wei, J.J., Xin, D.L., Chen, X., Quan, X.Y., Zhang, J.H., 2019. Effect of steam explosion on extraction of bioactive components and gutta-percha from barks of *Eucommia ulmoides Oliver*. Chem. Ind. For. Prod. 39 (01), 88–94.

Wells, T., Wei, Z., Ragauskas, A., 2015. Bioconversion of lignocellulosic pretreatment effflfluent via oleaginous *Rhodococcus opacus* DSM 1069. Biomass-.-. Bioenergy 72, 200–205.

Wen, L., Li, X.Y., 2021. Production of intracellular lipids from thermally hydrolyzed waste sludge by oleaginous yeast for energy and resource recovery. Energy Convers. Manag. 252, 115129.

Wu, Y.L., Peng, C.F., Cui, Z.J., Xiong, X., Xiao, D.F., Li, B., Yin, Y.L., 2021. Effects of yeast polysaccharide on growth performance, intestinal morphology and intestinal immune status of weaned piglets. Chin. J. Anim. Nutr. 33 (3), 1349–1358.

Xia, L., Gao, H., Geng, J.T., 2019. Facile fabrication of foamed natural *Eucommia ulmoides* gum composites with heat-triggered shape memory behavior. Polym. Compos. 40 (8), 3075–3083.

Xu, J.M., Xie, X.F., Wang, J.X., Jiang, J.C., 2016. Directional liquefaction coupling fractionation of lignocellulosic biomass for platform chemicals. Green. Chem. 18 (10), 3124–3138.

Yang, F., Hu, S.R., Li, D.H., Kang, H.L., Fang, Q.H., 2020. Progress in extraction methods of *Eucommia ulmoides* gum. Polym. Mater. Sci. Eng. 36 (4), 177–182, 190.

Yu, X., Zheng, Y., Xiong, X., Chen, S., 2014. Co-utilization of glucose, xylose and cellobiose by the oleaginous yeast *Cryptococcus curvatus*. Biomass-.-. Bioenergy 71, 340–349.

Zhan, J.J., Hong, Y., Hu, H.Y., 2016. Effects of nitrogen sources and C/N ratios on the lipid-producing potential of *Chlorella sp*. HQ. J. Microbiol. Biotechnol. 26 (7), 1290–1302.

Zhang, L., Lee, J.T.E., Yong, S.O., Dai, Y.J., Tong, Y.W., 2022. Enhancing microbial lipids yield for biodiesel production by oleaginous yeast *Lipomyces starkeyi* fermentation: A review. Bioresour. Technol. 2022 144, 126294.

Zhang, X.J., Gong, B.H., Wang, Q.H., Han, S.F., Cheng, C., 2009. Hydrolysis of plant cell wall of Eucommia ulmoides by cellulase and extraction of long silk gum. Nat. Prod. R. D. 21 (1), 115–121.

Zhao, C., Chen, S.L., Fang, H., 2018. Consolidated bioprocessing of lignocellulosic biomass to itaconic acid by metabolically engineering Neurospora crassa. Appl. Microbiol. Biotechnol. 102, 9577–9584.

Zhao, Z., Xian, M., Liu, M., Zhao, G., 2020. Biochemical routes for uptake and conversion of xylose by microorganisms. Biotechnol. Biofuels 13, 21.

Zheng, Y., Chi, Z., Ahring, B.K., Chen, S., 2012. Oleaginous yeast *Cryptococcus curvatus* for biofuel production: ammonia's effect. Biomass Bioenergy 37, 114–121.

Zhou, L.M., Zhang, Y., 2015. Study on the phenol-sulfuric acid determination method of alkali-insoluble yeast cell wall polysaccharides. Sci. Technol. Food Ind. 36 (3), 311–315.