Bioresource Technology 384 (2023) 129345

Contents lists available at ScienceDirect

Bioresource Technology

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

Coproduction of single cell protein and lipid from lignocellulose derived carbohydrates and inorganic ammonia salt with soluble ammonia recycling

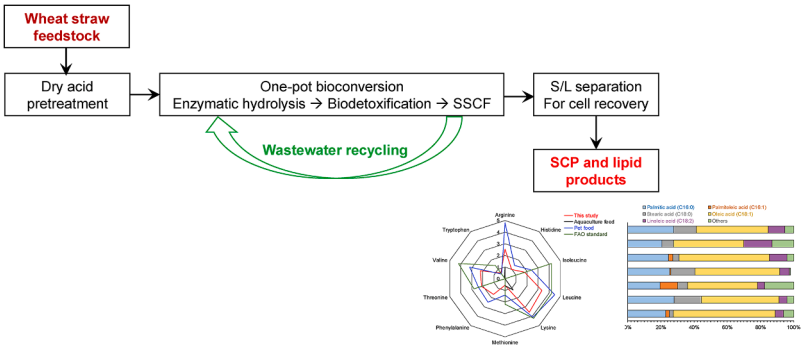
Bin Zhang [a,1], Dayu Ren [a,1], Qi Liu [a,1], Xiucai Liu [b], Jie Bao [a,*]

[a] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China
[b] Cathay Biotech Inc, 1690 Cailun Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China

HIGHLIGHTS

- SCP and lipid were coproduced from wheat straw and $(NH_4)_2SO_4$ by oleaginous yeast.
- Engineered *Trichosporon cutaneum* MP11 with excellent adaptability was employed.
- The cellulosic SCP and lipid titer reached 24.4 ± 1.5 g/L and 11.8 ± 1.2 g/L at 48 h.
- Residual $(NH_4)_2SO_4$ in wastewater was recycled with stable fermentation performance.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Single cell protein
Microbial lipid
Lignocellulose
Trichosporon cutaneum
Wastewater

ABSTRACT

Co-production of single cell protein (SCP) and lipid from lignocellulose-derived carbohydrates and inorganic ammonia offers a promising alternative for poultry or aquaculture feeds. An engineered oleaginous yeast *Trichosporon cutaneum* MP11 showed great potential for producing SCP and lipid from wheat straw and ammonia sulfate with minimum nutrient input. *Trichosporon cutaneum* MP11 showed stronger SCP and lipid fermentability using dry acid pretreated and biodetoxified wheat straw than using pure sugars. The residual ammonium sulfate in fermentation broth was recycled up to five times, resulting in ~70% of nitrogen fixation into SCP. The overall yield of SCP and lipid from lignocellulose-derived sugars was 0.15 g/g and 0.11 g/g, respectively. This translates to the production of one ton of SCP (0.56 ton) and lipid (0.44 ton) from 6.6 tons of wheat straw, or one ton of SCP and lipid containing yeast cells (dry) from 4.8 tons of wheat straw.

1. Introduction

Protein and lipid are essential components of animal diet and nutrients (Bajic et al., 2023; Ghazani & Marangoni, 2022). Single cell protein (SCP) is a protein-rich biomass produced by bacteria, yeast, fungi, and algae (Tian et al., 2023). Microbial lipid generally is also produced and harvested along with SCP during SCP production (Wang et al., 2020). SCP and microbial lipid can serve as alternative sources of

* Corresponding author.
E-mail address: jbao@ecust.edu.cn (J. Bao).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.biortech.2023.129345
Received 29 May 2023; Received in revised form 14 June 2023; Accepted 15 June 2023
Available online 20 June 2023

protein and lipid to animal- or plant-derived products for both humans and animals (Jones et al., 2019; Ritala et al., 2017). Among these microorganisms, yeast is a highly promising cell factory for SCP production due to its fast growth rate, high protein content, good flocculation ability, pleasant taste, and lack of pathogenicity risk (Kieliszek et al., 2017). The feedstocks used in SCP production include carbohydrates and ammonia compounds as carbon and nitrogen sources, which typically account for approximately 75% of the total production costs (Sharif et al., 2021). The use of cheap agro-industrial lignocellulosic biomass as carbohydrate feedstocks such as corn stover, wheat straw, rice straw, or sugarcane bagasse is an important option to reduce the overall cost of SCP production (Jin et al., 2015; Thiviya et al., 2022b). The reasonable nitrogen source should be derived from cheap inorganic ammonia compounds or high ammonia-containing liquid wastes (Singh et al., 2022; Wada et al., 2022). The agricultural wastes such as wheat straw, corn stalk, rice straw, have been reported for SCP production (Yang et al., 2022). However, the saccharification and fermentation were generally conducted at low solids loading (lower than 10%, w/w), which limited the fermentation efficiency.

Microbial cell factories for SCP production using lignocellulosic feedstock should be tolerant to inhibitors generated in pretreatment step (Sitepu et al., 2014), and be able to assimilate lignocellulose-derived sugars (especially for non-glucose monosaccharides) (Ko & Lee, 2018; Vasconcelos et al., 2019). Adequate intracellular space is also required to accumulate a high amount of SCP. Oleaginous yeast is a preferred cell factory due to its excellent adaptability to lignocellulosic hydrolysate and the capacity to accumulate intracellular products. The lipid produced by oleaginous yeast adds a new source into SCP as a two-nutrient product of food or feed (Chen et al., 2021).

An engineered oleaginous yeast *Trichosporon cutaneum* MP11 had been developed in previous studies showed great potentials to coproduce SCP and lipid from wheat straw due to its excellent adaptability to lignocellulosic hydrolysate, complete and coordinate assimilations of all non-glucose monosaccharides derived from lignocellulose, enhanced lipid synthesis, and enlarged intracellular volume (Liu et al., 2022). However, the first new challenge for oleaginous yeasts is the switch of nitrogen limitation required for lipid accumulation (high C/N ratio) into nitrogen excess required for SCP synthesis (low C/N ratio) (Bharathiraja et al., 2017; Kumar et al., 2017; Salazar-López et al., 2022; Sharif et al., 2021). The excessive nitrogen addition for SCP fermentation leads to the generation of high ammonia-containing wastewater after the fermentation. The effective recycling of high ammonia-containing wastewater forms the second new challenge.

In this study, the conversion of lignocellulosic feedstock and inorganic nitrogen to SCP and lipid by *T. cutaneum* MP11 was investigated at high solids loading (~24%, w/w). The residual ammonium sulfate in the fermentation broth was reutilized by recycling the cell-free fermentation supernatant for at least five recycles to minimize the ammonia-containing wastewater generation. This study proposed an efficient integrative biorefinery process for coproduction of SCP and lipid by engineered oleaginous yeast *T. cutaneum*.

2. Materials and methods

2.1. Feedstock and cellulase

Raw wheat straw was harvested from Nanyang City, Henan Province, China in 2021. The main compositions of raw wheat straw were determined by the two-step acid hydrolysis method (Sluiter et al., 2012), which include 31.2% of cellulose, 24.3% of xylan, 19.4% of lignin, and 9.6% of ash on dry weight base (w/w).

The cellulase Cellic CTec2.0 was purchased from Novozymes (Beijing, China). The protein content of Cellic CTec2.0 was determined to be 87.3 mg/mL by Coomassie brilliant blue staining (Bradford, 1976). The initial activity of Cellic CTec2.0 was 256.1 FPU/mL.

2.2. Strains and medium

The SCP and lipid coproducing strain *T. cutaneum* MP11 (CGMCC 20481) was obtained by long-term evolution from the parental strain *T. cutaneum* ACCC 20271 (Liu et al., 2022). *T. cutaneum* MP11 was preserved on yeast extract peptone dextrose (YPD) plate. The nutrients for cellulosic SCP and lipid coproduction by *T. cutaneum* MP11 contain 1 g/L of KH_2PO_4, 1 g/L of $MgSO_4{\cdot}7H_2O$, and 24 g/L of $(NH_4)_2SO_4$.

Paecilomyces variotii FN89 (CGMCC 17665) was isolated from acid pretreated biomass and applied for the biodetoxification of wheat straw hydrolysate (Zhang et al., 2021a). *P. variotii* FN89 was preserved on potato dextrose agar (PDA) plate and cultured in synthetic medium composed of 2 g/L of KH_2PO_4, 1 g/L of $MgSO_4{\cdot}7H_2O$, 1 g/L of yeast extract, 1 g/L of $(NH_4)_2SO_4$, and 20 g/L of glucose.

2.3. Pretreatment, hydrolysis and biodetoxification

The raw wheat straw was pretreated by dilute acid pretreatment according to the method described by Liu et al. (2018). The pretreated wheat straw was neutralized by adding $CaCO_3$. The enzymatic hydrolysis was conducted by loading all the pretreated wheat straw solids up to 30% (w/w) of the total hydrolysate into the 5-L bioreactor equipped with a helical impeller within 2 h. The wheat straw feedstock maintained as solids form after dry acid pretreatment. The wheat straw solids were gradually loaded into the reactor with cellulase enzyme and hydrolyzed till the solids were converted into liquid slurry form. The whole enzymatic hydrolysis period was lasted for 12 h.

P. variotii FN89 was cultured in synthetic medium at 37 °C, 300 rpm for 18 h, and then inoculated into the hydrolysate at the inoculum ratio of 10% (w/w) to remove the inhibitors such as acetic acid, furfural, and 5-hydroxymethylfurfural (HMF). The biodetoxification was conducted at 37 °C, 750 rpm, and 1 vvm for 13 h. The biodetoxification strain *P. variotii* FN89 in hydrolysate was then inactivated by boosting the temperature to 50 °C and maintaining for 12 h anaerobically (Zhang et al., 2021b). The bioreactor was equipped with the condenser in the air release port to prevent the water evaporation.

2.4. Simultaneous saccharification and co-fermentation (SSCF) and wastewater recycling

The feasibility of coproduction of SCP and lipid by *T. cutaneum* MP11 was tested using lignocellulose-derived sugars and inorganic nitrogen compounds. Ammonium sulfate was selected as the cheap inorganic nitrogen source as the literature reported that ammonium sulfate was commonly used for SCP production (Salazar-López et al., 2022). The raw wheat straw was dry acid pretreated, enzymatically hydrolyzed, biodetoxified, and then used for SSCF. The initial wheat straw hydrolysate contained ~120 g/L of fermentable sugars mixture, including ~80 g/L of glucose and ~40 g/L of xylose.

One colony of *T. cutaneum* MP11 on YPD plate was picked up and inoculated into 20 mL of YPD medium in 100 mL flask, cultured at 30 °C, 180 rpm for 24 h. Then the culture broth was transferred into 50 mL of YPD medium with 10% (v/v) inoculum ratio in 500 mL flasks, and cultured at 30 °C, 180 rpm for 24 h as the seed. The seed was inoculated into the biodetoxified wheat straw hydrolysate at the ratio of 10% (w/w). The inoculation of biodetoxification microbe seed and the fermentation yeast seed at the inoculum of 10% (w/w) reduced the total solids loading by approximately 3% (w/w), respectively. The SSCF was conducted at 30 °C, 600 rpm, 1.0 vvm. The fermentation pH was controlled by automatically adding 2 M H_2SO_4 solution and 5 M NaOH solution.

The fermentation broth was centrifuged at 8,000 rpm to remove the cells and inert lignin solid fractions. The liquid supernatant contained approximately 7.6 g/L of residual ammonium sulfate. Then the supernatant was used to adjust the solids content to 30% (w/w) of the total wheat straw, and to prepare the medium for seed culture of *T. cutaneum* MP11. Ammonium sulfate was re-supplemented in the wheat straw

hydrolysate till the C/N ratio reached 9.4 for the next round of SCP and lipid fermentation. Totally five runs of recycling were performed.

2.5. Clarified wheat straw hydrolysate preparation

Wheat straw was pretreated, biodetoxified, and enzymatically saccharified for 48 h into hydrolysate slurry. Then the slurry was centrifuged at 8,000 rpm for 10 min to remove solids. The supernatant was collected, sterilized and filtrated to obtain clarified wheat straw supernatant, which is similar with pure sugar medium.

2.6. Lipid and SCP determination

For the determination of lipid and SCP production, the fermentation broth was centrifuged at 8000 rpm for 10 min. The solid fraction was air-dried at 65 °C to constant weight. Lipids were extracted by chloroform–methanol method after the lysis of cells by adding 4 M HCl (Liu et al., 2022).

The protein content and SCP titer were determined by semi-automatic Kjeldahl apparatus (PeiOu Analysis Instrument, Shanghai, China). For the fermentation in clarified wheat straw hydrolysate, the single-cell protein content (w_1, %) was calculated according to Eq. (1) as follows:

$$\text{Protent content } \% : w_1 = \frac{[c] \times ([V_i] - [V_0]) \times 14 \times 6.25}{m_1} \times 100\% \quad (1)$$

where [c] (mol/L) is the concentration of HCl for titration, which was 0.05 mol/L in this study; [V_i] (mL) is the usage of HCl for titration; [V_0] (mL) is the usage of hydrochloric acid for titration of blank control; 14 is the molar mass of nitrogen; 6.25 is the coefficient for conversion of nitrogen content to protein content; m_1 (g) is the dry cell weight.

The SCP titer (c_1, g/L) in clarified wheat straw hydrolysate was calculated according to Eq. (2) as follows:

$$\text{SCP titer g/L} : c_1 = \frac{W_1 \times m_1}{V} \quad (2)$$

where w_1 (%) is the single-cell protein content; m_1 (g) is the dry cell weight; V (mL) is the broth volume.

For the SSCF, the single-cell protein content cannot be calculated owing to the solid fraction mixture including not only cell biomass but also lignin residues, ash, et al. The nitrogen content (w_2, %) of solid fraction in SSCF was calculated according to Eq. (3) as follows:

$$\text{Nitrogen content } \% : w_2 = \frac{[c] \times ([V_i] - [V_0]) \times 14}{m_2} \times 100\% \quad (3)$$

where m_2 is the mass of dried solid fraction.

The produced SCP content (w, %) in solid fraction of SSCF was calculated according to Eq. (4) as follows:

$$\text{Produced SCP content } (\%) : w = 6.25 \times (w_2 - w_3 - w_4) \quad (4)$$

where w_3 is the nitrogen content of solid fraction without digestion; w_4 is the nitrogen content of solid fraction at the beginning of fermentation.

The SCP titer (c_2, g/L) in SSCF was calculated according to Eq. (5) as follows:

$$c_2 = \frac{w \times m_2}{V} \quad (5)$$

where w (%) is the produced SCP content in solid fraction; m_2 (g) is the mass of dried solid fraction; V (mL) is the broth volume.

2.7. Lipid and SCP evaluations

The extracted lipid was esterified and transformed into fatty acid methyl ester. The fatty acid composition of lipid was determined by chromatography-mass spectrometry (GC–MS) (Hu et al., 2018). The column was Agilent 19091 J-433 (30 m × 250 μm × 0.25 μm) with no split injection. The injection port temperature was at 280 °C. Injection volume was 0.4 μL. Helium was used as carrier gas with the flow rate of 1 mL/min. The temperature of flame ionization detector (FID) was set at 120 °C. The temperature was maintained at 80 °C for 3 min, then increased to 280 °C at a rate of 16 °C/min and maintained for 8 min. The analysis system was NITS MS Search 2.0.

The nucleic acid was extracted from the collected SCP according to the previously reported method (Moreno et al., 1991). The nucleic acid content was measured at 260 nm using NanoDrop ND-100 spectrophotometer.

The collected SCP was freeze-dried overnight and then hydrolyzed (Biswas et al., 2020). The amino acid content was determined using Hitachi (Japan) L-8900 Amino Acid Analyzer equipped with 2622#PH ion exchange column.

2.8. Analytical methods

Glucose, xylose, acetic acid, HMF, and furfural were determined by high-performance liquid chromatography (HPLC) using Shimadzu system equipped with RID-10A detector and Bio-rad Aminex HPX-87H column (Zhang et al., 2021a). The main elements' content was measured by inductively coupled plasma atomic emission spectrometry (Han & Bao, 2018). The total phenolics in the broth were determined by Folin & Ciocalteu method with modifications according to the previous report (He et al., 2022). The qRT-PCR protocols were according to the methods described by Liu et al. (2022). Briefly, the total RNA of *T. cutaneum* MP11 cultured for 24 h or 48 h was extracted using Trizol reagent (RNAiso Plus, TAKARA). The reverse transcription reaction was performed using ReverTra Ace quantitative real-time polymerase chain reaction (qPCR RT) Master Mix with gDNA Remover Kit (Toyobo). Each qRT-PCR reaction was performed using SYBR Green Real-time PCR Master Mix Kit (Toyobo) on a BioRad CFX 96. Actin gene was used as an internal control to normalize for difference in total RNA quantity.

3. Results and discussion

3.1. Assimilation of lignocellulose derived sugars and inorganic nitrogen for coproduction of single cell protein and lipid

The optimal C/N ratio for coproduction of SCP and lipid by *T. cutaneum* MP11 was examined by adding 12 g/L, 24 g/L, 36 g/L, and 48 g/L of ammonium sulfate to the hydrolysate, equivalent to the C/N ratio of 18.8, 9.4, 6.3, and 4.7, respectively (Fig. 1). The complete assimilation of lignocellulose-derived glucose and xylose was achieved within 72 h for all the cases (Fig. 1a and 1b). The consumption of xylose was simultaneously utilized with glucose at the C/N ratio of 9.4, but slightly lagged at higher (18.8) or lower (4.7) C/N ratios. The maximum SCP titer of 24.4 ± 1.5 g/L was obtained at 48 h and C/N ratio of 9.4. Higher or lower C/N ratios showed negative effects on SCP production (Fig. 1c). A phenomenon was observed that the titer of SCP decreased from 48 h, perhaps owing to the nutrient depletion triggered the consumption of SCP by the yeast cells (Gervasi et al., 2018).

The lipid titer increased with the elevated C/N ratios (Fig. 1d), which is consistent with the nitrogen limitation for lipid accumulation in oleaginous yeasts (Ko & Lee, 2018). The maximum lipid titer reached 18.3 ± 0.1 g/L at 72 h with C/N ratio of 18.8. Different from the trend of SCP production, the lipid titer stably increased during the fermentation, perhaps due to (i) the utilization of ammonia for SCP synthesis reduced the ammonia concentration and led to a nitrogen limitation favorable for lipid synthesis; (ii) the abundance of non-glucose sugars (xylose, arabinose, mannose, and galactose) and organic acids (e.g., acetic acid, levulinic acid) in the hydrolysate were capable for lipid synthesis, but unable for SCP synthesis (Wang et al., 2016).

The optimal pH for coproduction of SCP and lipid by *T. cutaneum*

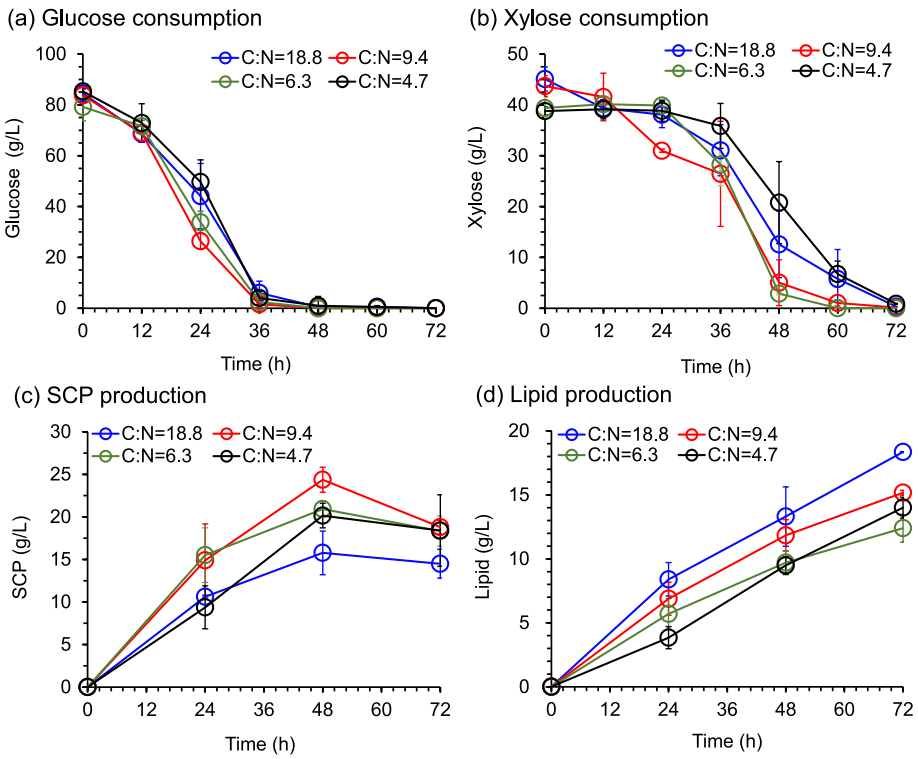


Fig. 1. SCP and lipid production from biodetoxified wheat straw hydrolysate at different C/N ratios. (a) Glucose consumption; (b) xylose consumption; (c) SCP production; (d) lipid production. SSCF conditions: ~24% (w/w) solids loading, 4 mg cellulase protein/g dry matter, 30 °C, 600 rpm, 1.0 vvm, pH 5.0.

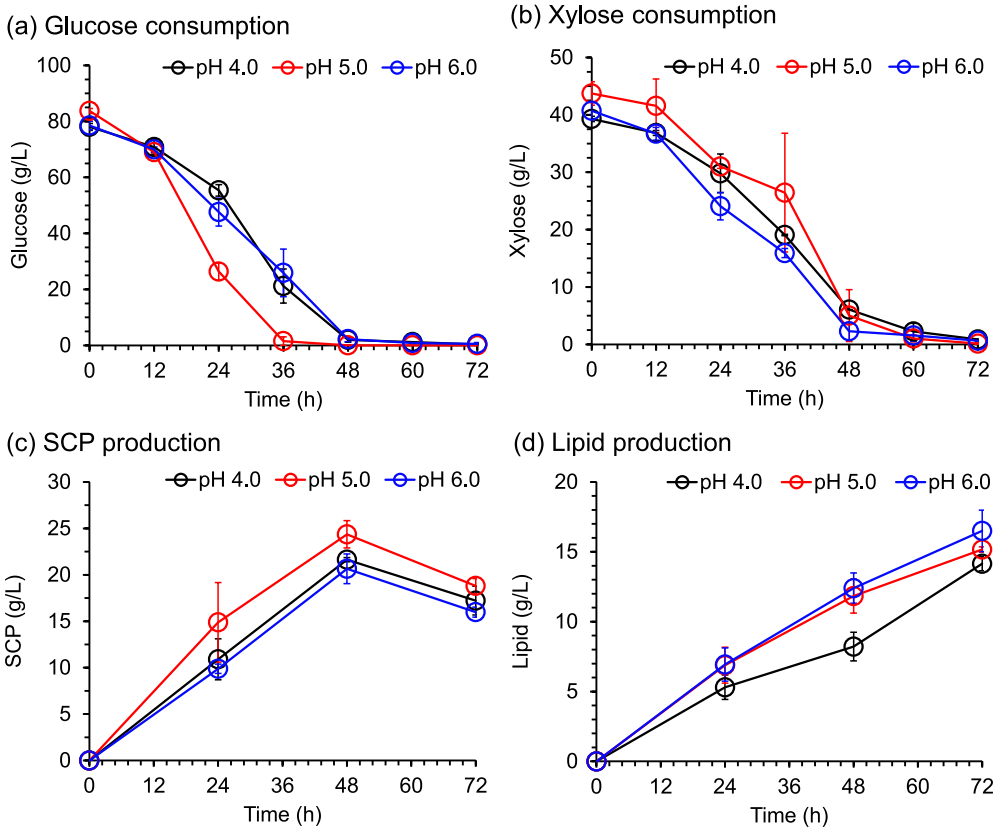


Fig. 2. SCP and lipid production from biodetoxified wheat straw hydrolysate at different pH values. (a) Glucose consumption; (b) xylose consumption; (c) SCP production; (d) lipid production. SSCF conditions: C/N ratio of 9.4 (24 g/L of ammonium sulfate), ~24% (w/w) solids loading, 4 mg cellulase protein/g dry matter, 30 °C, 600 rpm, 1.0 vvm.

MP11 was further examined in the range of commercial cellulase activity (Fig. 2). The consumption of glucose was accelerated at pH of 5.0 (Fig. 2a), but the pH varying had no significant effect on xylose consumption (Fig. 2b). Kot et al. (2017) reported that the initial pH in the range of 4.0–7.0 did not have a significant influence either on cell growth, lipid content, or protein content of *Rhodotorula glutinis*. Tian et al. (2023) reported that the pH of ~4.0 was optimal to obtain a better SCP titer for yeast consortium. Fig. 2c and 2d showed a maximum of SCP at pH 5.0, while the lipid titer also maintained high at pH 5.0.

The results suggest that a balance was achieved between SCP production under high nitrogen content condition and lipid production under nitrogen limitation condition, resulting in satisfactory yields of both SCP and lipid using lignocellulose-derived carbohydrates and inorganic ammonia nitrogen. The coordinated sugars assimilation and intracellular product accumulation contributed to the efficient coproduction of SCP and lipid by *T. cutaneum* MP11. Totally 24.4 ± 1.5 g/L of SCP and 11.8 ± 1.2 g/L of lipid were produced by *T. cutaneum* MP11 at C/N ratio of 9.4, pH 5.0 within 48 h from 24 g/L of ammonium sulfate and ~24% (w/w) solids loading biodetoxified wheat straw hydrolysate.

3.2. Wheat straw and refined sugars as feedstocks for coproduction of SCP and lipid

Coproduction of SCP and lipid using lignocellulose-derived sugars were compared with that using refined sugars (Fig. 3). To conduct the comparative experiment, wheat straw was prepared into clarified hydrolysate, which is similar with pure sugar medium. The cell growth, single-cell protein, and lipid content were measured in both refined sugars-containing synthetic medium and clarified wheat straw hydrolysate.

Fig. 3a shows that glucose and xylose were completely consumed by *T. cutaneum* MP11 in clarified hydrolysate within 96 h, but only 66.1% of glucose and 9.5% of xylose in synthetic medium were utilized. The greater sugar utilization in clarified hydrolysate promoted the cell growth to 76.7 ± 5.9 of OD at 600 nm at 96 h, which was 1.9 folds greater than that (41.1 ± 1.8) in synthetic medium (Fig. 3b). The SCP production using wheat straw feedstock reached the maximum (10.7 ± 0.9 g/L) at 72 h, which was 2.6 folds greater than that (4.1 ± 0.3 g/L) using refined sugars. The protein content in cells reached the maximum at 24 h (36.7% ± 0.7% *vs.* 37.7% ± 0.7%) in both synthetic medium and clarified hydrolysate, then declined after 24 h (SCP content in synthetic medium showed a more rapid decrease than that in clarified hydrolysate) (Fig. 3c). Zayed & Mostafa (1992), and Thiviya et al. (2022a) attributed this phenomenon to that the mature cells (after 24 h of growth) would switch to storing high amounts of carbohydrates. Kumar et al. (2017) further explained that the lipid accumulation in certain oleaginous yeasts occurred at the expense of proteins and nucleic acids under the limited availability of nitrogen in lignocellulosic hydrolysate. On the other side, this result is in accordance with the reported finding by (Yang et al., 2022) that *Saccharomyces cerevisiae* is more favorable to accumulate SCP in corn stover hydrolysate than that in synthetic medium.

The lipid production in clarified hydrolysate was lower than that in synthetic medium within 48 h (Fig. 3d), probably due to the negative effect of weak organic acids and phenolic aldehyde inhibitors on lipid accumulation (Liu et al., 2020; Zhang & Bao, 2022). However, with the gradual degradation of the inhibitors (Wang et al., 2016) and the decrease of nitrogen concentration at the latter stage (from 48 to 96 h), the sugars consumption, cell growth, and lipid production of *T. cutaneum* MP11 were improved in clarified hydrolysate (Fig. 3a, 3b, and 3d). Finally, 15.9 ± 0.1 g/L and 23.0 ± 0.6 g/L of lipid were produced at 72 h and 96 h in clarified hydrolysate, which was 1.4 and 1.6 folds greater

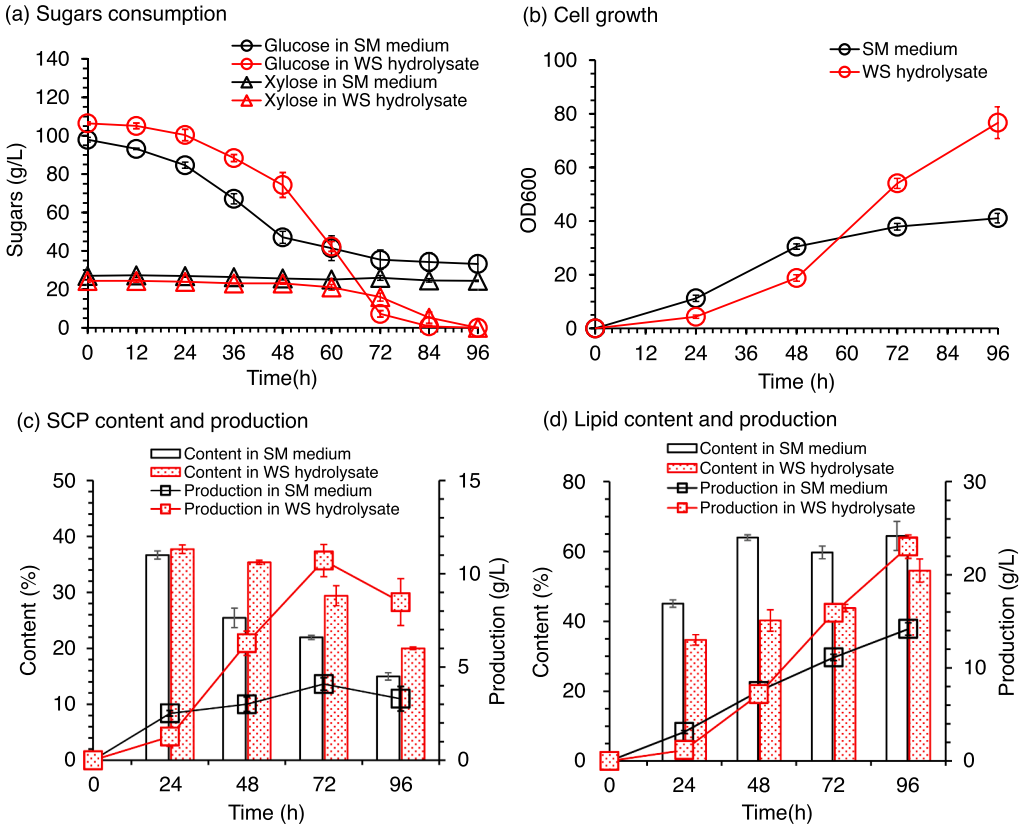


Fig. 3. Comparison of SCP and lipid production between clarified wheat straw hydrolysate and in thetic medium. (a) Sugars consumption; (b) cell growth; (c) SCP production and single-cell protein content; (d) lipid production and single-cell lipid content. Conditions: C/N ratio of 9.4 (24 g/L of ammonium sulfate), 30 °C, 600 rpm, 1.0 vvm, pH 5.0. SM, synthetic medium; WS, wheat straw.

than that in synthetic medium, respectively (Fig. 3d).

These results indicated that *T. cutaneum* MP11 is well-adapted to lignocellulosic hydrolysate containing high concentration of inorganic ammonia–nitrogen for coproduction of SCP and lipid. The coproduction of SCP and lipid using lignocellulose-derived sugars as carbon source are superior to that using pure sugars in respect of the sugar consumption rate, cell growth rate, and SCP and lipid production levels by *T. cutaneum* MP11.

The relative transcriptional expression levels of the genes in ammonia assimilation, amino acid metabolism, sugar metabolism, and lipid synthesis pathways of *T. cutaneum* MP11 in wheat straw hydrolysate were analyzed and compared with those in synthetic medium (Fig. 4). The two timepoints of 24 h and 48 h were selected for the transcriptional analysis. Most of the genes in *T. cutaneum* MP11 related to amino acid metabolism and aminoacyl tRNA synthase were up-regulated in wheat straw hydrolysate, which facilitated the SCP synthesis. The glucose metabolism related genes did not show significant changes (data not shown), but Trcu-01943 encoding D-xylose reductase for reduction of D-xylose to D-xylulose (Ishizaki & Hasumi, 2014) was significantly up-regulated by 4.1 and 3.7 folds at 24 h and 48 h, respectively. The up-regulation of D-xylose reductase contributes to the efficient utilization of xylose by *T. cutaneum* MP11 when lignocellulose-derived sugars are used as the substrates. At 48 h, Trcu-03536 encoding diacylglycerol O-acyltransferase in triglyceride synthesis was up-regulated by 2.3-folds (Fig. 4b), contributing to the higher accumulation of lipid in wheat straw hydrolysate. The differential transcriptional expression in wheat straw hydrolysate resulted in the increased efficiency of lignocellulose-derived sugars conversion and synthesis for both SCP and lipid.

3.3. Utilization of residual ammonium in fermentation broth by cell-free wastewater recycling

SCP production should be a nitrogen assimilation process, instead of being a process with extra ammonium generation in discharged wastewater (Wada et al., 2022). This study suggests that one ton of SCP and lipid production generates 5–10 tons of fermentation broth containing ~7.6 g/L of residual ammonium sulfate. According to the National Standard of China for integrated wastewater discharge (GB 8978–1996, https://www.mee.gov.cn/), the ammonia nitrogen concentration in industrial effluents should not exceed 50 mg/L at maximum. The treatment of fermentation wastewater would generate high technical and economic burdens for SCP and lipid production.

The attempt to utilize the residual ammonium sulfate was made by

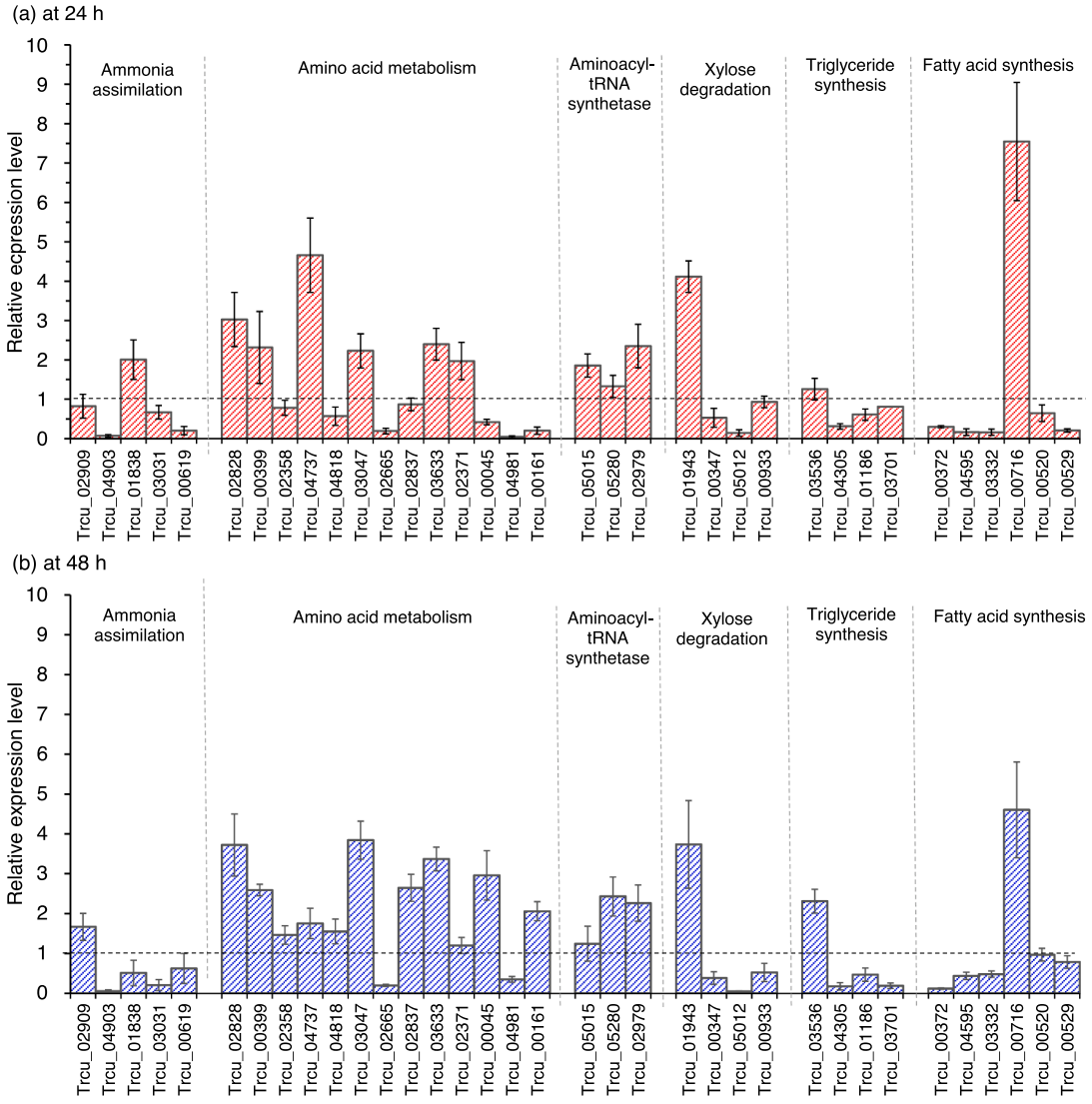


Fig. 4. Relative differential expressions of the key genes of *T. cutaneum* MP11 in clarified wheat straw hydrolysate compared with these in synthetic medium. (a) 24 h; (b) 48 h.

recycling the fermentation broth after the cells were harvested (Fig. 5). The supernatant from the solid/liquid separation of fermentation broth was directly used for wheat straw hydrolysis and seed culture of *T. cutaneum* MP11. Then the wheat straw hydrolysate was biodetoxified and supplemented with ammonium sulfate to C/N ratio of 9.4 for the next round of SCP and lipid fermentation. Totally five runs of recycling were performed.

The recycling of wastewater led to a certain decrease in sugar consumption rate, but eventually, all the glucose and xylose were utilized within 72 h (Fig. 5a–5b). The recycling of wastewater also caused the reduced and prolonged production of SCP from 24.4 ± 1.5 g/L SCP at 48 h in the first run to 18.5–20.2 g/L at 72 h in the five runs with wastewater recycling (Fig. 5c). The wastewater recycling showed less effect on lipid production (13.1 g/L to 17.0 g/L in the five runs) and no significant decrease compared with the first run without wastewater recycling (15.2 ± 0.2 g/L) (Fig. 5d). In the successive run cases, the overall sugars consumption, nitrogen conversion, SCP and lipid titers, yields, and productivities gradually became stable (Table 1). The nitrogen conversion ratio from ammonium sulfate to SCP was ~70%. The yield from the fermentable sugars to SCP and lipid was ~0.15 g/g and ~0.11 g/g, respectively, which was close to the yield in synthetic medium (glucose as carbon source) reported previously (Wang et al., 2020).

The major element contents including Ca, Mg, P, K, Na, and S were measured and no significant changes were observed, indicating the recycling of wastewater did not cause salt ions accumulation (Fig. 5e). The wastewater contained higher contents of Na and S, mainly due to the use of neutralizers and ammonium sulfate. The excellent salt tolerance of *T. cutaneum* MP11 (up to 130 g/L of NaCl) should help the yeast cells to be survived from the possible salt accumulation (Sun et al., 2021). Therefore, the salt ions in the wastewater only had minor effects on the fermentability of *T. cutaneum* MP11.

The total phenolics concentration in the wastewater increased consistently with the repeated recycling of wastewater (Fig. 5f). The total phenolics concentration in the fifth recycling reached more than 900 mg/L, which was approximately 30% higher than that in the initial wastewater recycling. It is expected that the total phenolics concentration would keep rising if the recycling of wastewater continues. Although *T. cutaneum* strain had better tolerance to lignocellulose-derived phenolic inhibitors than other oleaginous yeasts (Zhang & Bao, 2022), the consistently accumulated higher concentrations of phenolics in water would inhibit the cell growth, SCP, and lipid coproduction of *T. cutaneum* MP11.

3.4. Product evaluation and overall mass balance

The evaluation of SCP and lipid produced from wheat straw by *T. cutaneum* was performed. The nucleic acid content in SCP was determined to be 8.01% (w/w), which is within the regular nucleic acid content of fungal-produced SCP (Ritala et al., 2017). However, the nucleic acid content may be too high for human food use and an additional process may be needed to reduce it. The essential amino acid concentrations (%, w/w) of SCP produced from wheat straw were shown in Fig. 6a. The content was compared only with the standard contents of protein in different commercial feeds and food, while other typologies of nutrients such as carbohydrates, lipids, vitamins, and minerals were not included. The essential amino acids of SCP in this work are similar to or higher than the typical aminoacidic profiles of aquaculture feed, suggesting that the SCP produced from wheat straw can be considered as a component for aquaculture feed. On the contrary, SCP in this work presented deficits compared to pet food or FAO standard: the amino acid concentrations such as isoleucine and methionine were 2–4 times lower than the required levels.

The fatty acid compositions of cellulosic lipid produced by *T. cutaneum* MP11 were compared to the cellulosic lipid produced by other strains (Fig. 6b). These microbial lipids of oleaginous yeasts mainly consist of C16 to C18 fatty acids, which are favorable for biodiesel production (Singh et al., 2022). The lipid produced by *T. cutaneum* MP11 contained more unsaturated fatty acids components (Palmitoleic acid C16:1, 2.1%; Oleic acid C18:1, 61.5%; Linoleic acid C18:2, 5.0%). These unsaturated fatty acids can be transesterified by chemical or enzymatic methods to produce fatty acid alkyl esters (FAAE), a near equivalent of petroleum-based fuels (Bonturi et al., 2015; Xue et al., 2018). The previous transcriptional analysis showed the high-level expression of delta-12 fatty acid desaturase Trcu-00716 in *T. cutaneum* MP11 (Fig. 4), which may lead to the increased in unsaturated fatty acids content of lipid.

The overall mass balance involving biorefinery chain and wastewater recycling for cellulosic SCP and lipid coproduction was calculated (Fig. 6c). The mass balance was calculated based on the average experimental results in Fig. 5a–5d. The mass balance started from 100.00 kg of dry wheat straw containing 31.24 kg of cellulose and 24.31 kg of xylan. The raw wheat straw was pretreated at ~70% (w/w) solids loading using dilute sulfuric acid as the catalyst. Approximately 80% of xylan was hydrolyzed and most cellulose component was well preserved during the acid pretreatment. The wheat straw absorbed all the acid solution and condensate during the pretreatment without wastewater generation. The harsh pretreatment led to the generation of inhibitors including 1.94 kg of acetic acid, 0.23 kg of HMF, and 0.15 kg of furfural. The pretreated wheat straw was enzymatically hydrolyzed at 30% (w/w) solids loading after the neutralization, and total 30.28 kg of glucose was released. The hydrolysate containing high concentrations of fermentable sugars and inhibitors was then inoculated with *P. variotii* FN89. The biodetoxification strain *P. variotii* FN89 degraded these inhibitors before fermentable sugars consumption (Zhang et al., 2021b). The mass of free glucose was increased during the biodetoxification due to continuous enzymatic hydrolysis. The biodetoxified hydrolysate was inoculated with *T. cutaneum* MP11 for the coproduction of SCP and lipid. The fermentation broth was separated to obtain solid fraction and liquid fraction. The solid fraction (with the moisture of ~60%, w/w) was used for product (SCP and lipid) and lignin component recovery. The liquid fraction was recycled for saccharification and seed culture.

For the recycling of wastewater in detail, total 177.00 kg of wastewater containing 0.28 kg of inorganic nitrogen, equivalent to 1.32 kg of ammonium sulfate, was recycled for saccharification and seed culture of *T. cutaneum* MP11. Additional 8.39 kg of ammonium sulfate was supplemented at the beginning of the fermentation, leading to the C/N ratio of 9.4. Finally, 8.58 kg of SCP (containing 1.42 kg of nitrogen) was produced from 100 kg of wheat straw with the inorganic nitrogen from ammonium sulfate conversion rate of ~70%. Meanwhile, the coproduction of lipid was 6.61 kg. This translates to the production of one ton of SCP (0.56 ton) and lipid (0.44 ton) from 6.58 tons of wheat straw, or production of one ton of microbial cells from 4.80 tons of wheat straw according to 73.2% of intracellular product content (29.4% of protein content; 43.8% of lipid content, Fig. 3c, 3d). These results indicate a successful conversion of waste agriculture feedstock and inexpensive inorganic nitrogen into high value-added products by *T. cutaneum* MP11 while the wastewater discharge is strictly restricted.

4. Conclusions

T. cutaneum MP11 showed well adaptability to lignocellulosic hydrolysate. The titer of SCP and lipid reached 24.4 ± 1.5 g/L and 11.8 ± 1.2 g/L using wheat straw hydrolysate at C/N ratio of 9.4, pH 5.0 for 48 h. Total five recycles of wastewater were successfully performed. The nitrogen conversion rate was stable at ~70%. The yield from lignocellulose-derived sugars to SCP and lipid was ~0.15 g/g and ~0.11 g/g. Mass balance further demonstrated the successful conversion of waste agriculture feedstock and cheap inorganic nitrogen to high value-added products by *T. cutaneum* MP11 with strict restrictions on wastewater discharge.

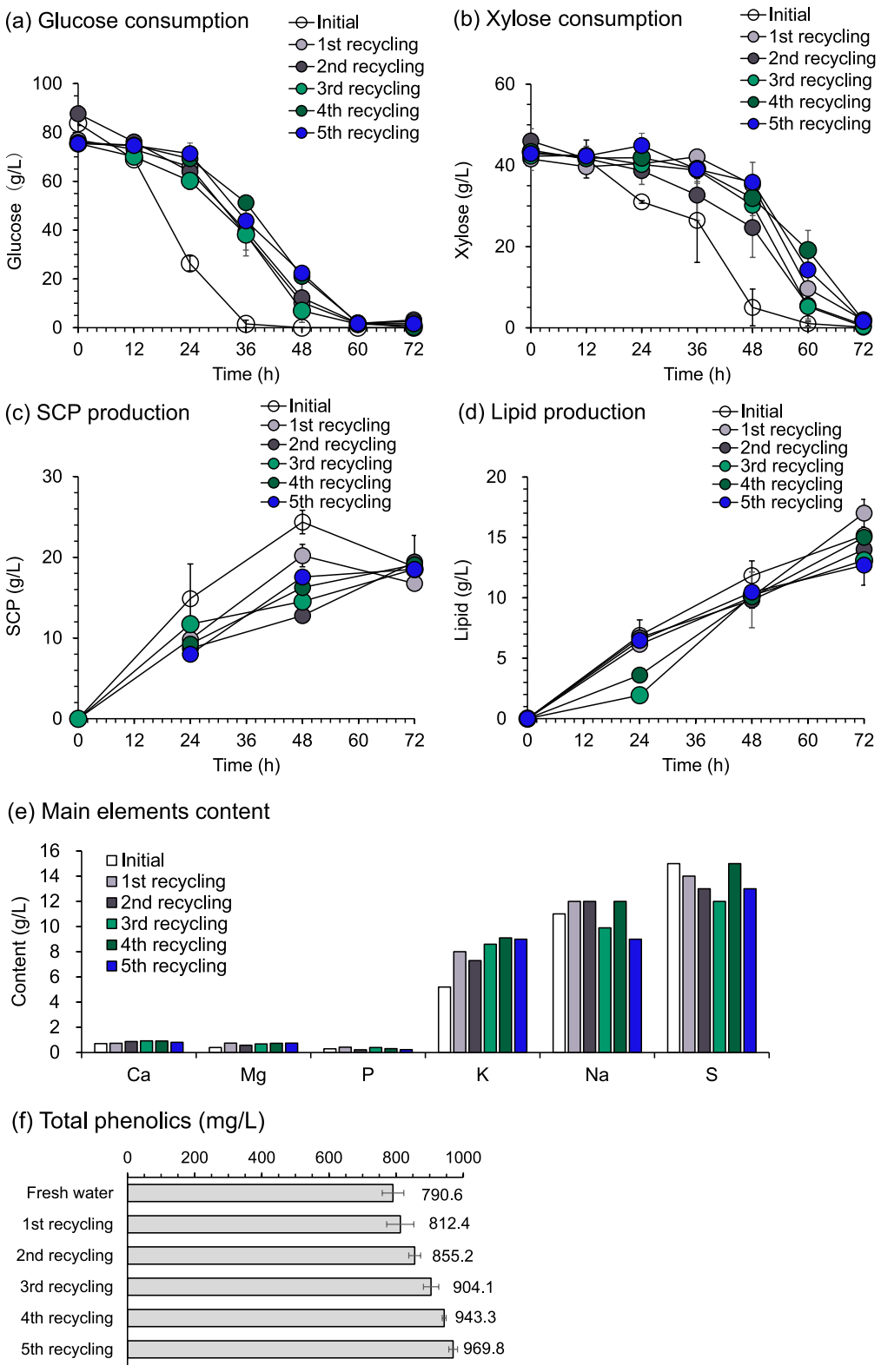


Fig. 5. Recycling of residual ammonia–nitrogen in fermentation broth. (a) glucose consumption; (b) xylose consumption; (c) SCP production; (d) lipid production; (e) main elements content in fermentation broth; (f) total phenolic concentration in fermentation broth. SSCF conditions: C/N ratio of 9.4, ~24% (w/w) solids loading, 4 mg cellulase protein/g dry matter, 30 °C, 600 rpm, 1.0 vvm, pH 5.0. Ammonium sulfate was added at the beginning of fermentation to reach the C/N ratio of 9.4.

Table 1
SCP and lipid coproduction performance from wheat straw with reusing the remaining inorganic ammonium in wastewater.

Cycles	Residual ammonium sulfate (g/L)	Single cell protein (SCP)			Microbial lipid	
		Nitrogen conversion (%)	Yield (g/g total sugars)	Productivity (g/L/h)	Yield (g/g total sugars)	Productivity (g/L/h)
Initial	7.6	71.5 ± 7.3	0.19 ± 0.02	0.51 ± 0.02	0.12 ± 0.01	0.22 ± 0.02
1st recycling	7.3	77.4 ± 3.1	0.16 ± 0.02	0.42 ± 0.02	0.13 ± 0.01	0.24 ± 0.02
2nd recycling	7.6	75.3 ± 17.1	0.15 ± 0.03	0.27 ± 0.02	0.11 ± 0.01	0.20 ± 0.02
3rd recycling	7.1	70.4 ± 4.8	0.14 ± 0.01	0.26 ± 0.02	0.10 ± 0.02	0.18 ± 0.04
4th recycling	6.7	71.4 ± 9.7	0.15 ± 0.02	0.26 ± 0.04	0.11 ± 0.01	0.21 ± 0.03
5th recycling	7.1	70.3 ± 1.5	0.14 ± 0.01	0.25 ± 0.01	0.11 ± 0.01	0.20 ± 0.02

Note: The time point for nitrogen conversion, yield and productivity calculations in the fresh water group and 1st recycling group was at 48 h; in other groups was at 72 h.

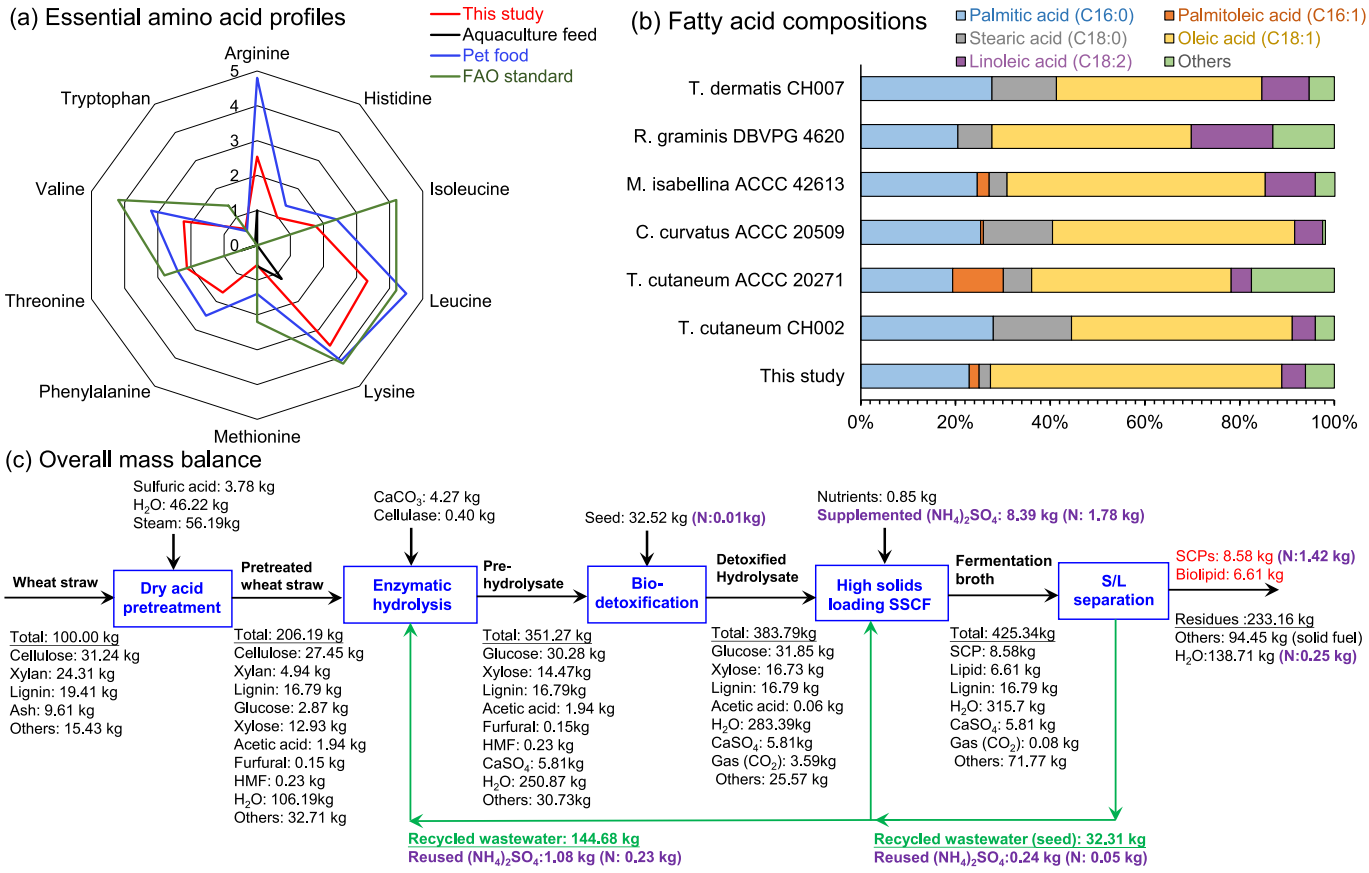


Fig. 6. Product evaluation and overall mass balance. Evaluation of the essential amino acid profile in SCP from wheat straw by T. cutaneum MP11 (a). Protein content values (w/w) are the mean of duplicate. The standard of aquaculture feed was referred to Nunes et al. (2014); the standard of pet food was referred to Beynen (2014); the standard of FAO was referred to Zhang & Zhao (2022). FAO, Food and Agriculture Organization of the United Nations. Fatty acid compositions of cellulosic lipid produced from wheat straw by T. cutaneum MP11 in this study compared to the cellulosic lipid produced by other strains (b). References: Huang et al. (2012); Galafassi et al. (2012); Ruan et al. (2012)); Gong et al. (2014); Wang et al. (2016); Chen et al. (2013). Overall mass balance of SCP and lipid coproduction from raw wheat straw with wastewater recycling (c). No wastewater or waste solids streams were generated during the overall biorefining process. Assumed there was no mass loss. The inorganic nitrogen mass balance was in purple; the wastewater mass balance was in green; the products of SCP and lipid were in red.

CRediT authorship contribution statement

Bin Zhang: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Dayu Ren:** Investigation, Visualization, Writing – original draft. **Qi Liu:** Data curation, Formal analysis, Writing – review & editing. **Xiucai Liu:** Funding acquisition, Resources. **Jie Bao:** Supervision, Project administration, Funding acquisition, Writing – review & editing.

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

This research was supported by the National Natural Science Foundation of China (21978083), and the Yangfan Project of Science and Technology Committee of Shanghai Municipality (23YF1409900).

References

Bajic, B., Vucurovic, D., Vasic, D., Jevtic-Mucibabic, R., Dodic, S., 2023. Biotechnological production of sustainable microbial proteins from agro-industrial residues and by-products. Foods. 12 (1), 107. https://doi.org/10.3390/foods12010107.

Beynen, A.C., 2014. Petfood label: analysis panel. Creature Companion 62–63.

Bharathiraja, B., Sridharan, S., Sowmya, V., Yuvaraj, D., Praveenkumar, R., 2017. Microbial oil - a plausible alternate resource for food and fuel application. Bioresour Technol. 233, 423–432. https://doi.org/10.1016/j.biortech.2017.03.006.

Biswas, A., Takakuwa, F., Yamada, S., Matsuda, A., Saville, R.M., LeBlanc, A., Silverman, J.A., Sato, N., Tanaka, H., 2020. Methanotroph (Methylococcus capsulatus, Bath) bacteria meal as an alternative protein source for Japanese yellowtail. *Seriola quinqueradiata*. Aquaculture. 529, 735700 https://doi.org/10.1016/j.aquaculture.2020.735700.

Bonturi, N., Matsakas, L., Nilsson, R., Christakopoulos, P., Miranda, E., Berglund, K., Rova, U., 2015. Single cell oil producing yeasts *Lipomyces starkeyi* and *Rhodosporidium toruloides*: selection of extraction strategies and biodiesel property prediction. Energies. 8 (6), 5040–5052. https://doi.org/10.3390/en8065040.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72 (1), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Chen, X.F., Huang, C., Yang, X.Y., Xiong, L., Chen, X.D., Ma, L.L., 2013. Evaluating the effect of medium composition and fermentation condition on the microbial oil production by *Trichosporon cutaneum* on corncob acid hydrolysate. Bioresour Technol. 143, 18–24. https://doi.org/10.1016/j.biortech.2013.05.102.

Chen, L., Qian, X., Zhang, X., Zhou, X., Zhou, J., Dong, W., Xin, F., Zhang, W., Jiang, M., Ochsenreither, K., 2021. Co-production of microbial lipids with valuable chemicals. Biofuels Bioprod Biorefining. 15 (3), 945–954. https://doi.org/10.1002/bbb.2209.

Galafassi, S., Cucchetti, D., Pizza, F., Franzosi, G., Bianchi, D., Compagno, C., 2012. Lipid production for second generation biodiesel by the oleaginous yeast *Rhodotorula graminis*. Bioresour Technol. 111, 398–403. https://doi.org/10.1016/j.biortech.2012.02.004.

Gervasi, T., Pellizzeri, V., Calabrese, G., Di Bella, G., Cicero, N., Dugo, G., 2018. Production of single cell protein (SCP) from food and agricultural waste by using *Saccharomyces cerevisiae*. Nat Prod Res. 32 (6), 648–653. https://doi.org/10.1080/14786419.2017.1332617.

Ghazani, S.M., Marangoni, A.G., 2022. Microbial lipids for foods. Trends Food Sci Technol. 119, 593–607. https://doi.org/10.1016/j.tifs.2021.10.014.

Gong, Z., Shen, H., Yang, X., Wang, Q., Xie, H., Zhao, Z.K., 2014. Lipid production from corn stover by the oleaginous yeast *Cryptococcus curvatus*. Biotechnol Biofuels. 7 (1), 158. https://doi.org/10.1186/s13068-014-0158-y.

Han, X., Bao, J., 2018. General method to correct the fluctuation of acid based pretreatment efficiency of lignocellulose for highly efficient bioconversion. ACS Sustain. Chem. Eng. 6 (3), 4212–4219. https://doi.org/10.1021/acssuschemeng.7b04601.

He, N., Jia, J., Qiu, Z., Fang, C., Liden, G., Liu, X., Bao, J., 2022. Cyclic l-lactide synthesis from lignocellulose biomass by biorefining with complete inhibitor removal and highly simultaneous sugars assimilation. Biotechnol Bioeng. 119, 1903–1915. https://doi.org/10.1002/bit.28082.

Hu, M., Wang, J., Gao, Q., Bao, J., 2018. Converting lignin derived phenolic aldehydes into microbial lipid by *Trichosporon cutaneum*. J. Biotechnol. 281, 81–86. https://doi.org/10.1016/j.jbiotec.2018.06.341.

Huang, C., Chen, X.F., Xiong, L., Chen, X.D., Ma, L.L., 2012. Oil production by the yeast *Trichosporon dermatis* cultured in enzymatic hydrolysates of corncobs. Bioresour Technol. 110, 711–714. https://doi.org/10.1016/j.biortech.2012.01.077.

Ishizaki, H., Hasumi, K., 2014. Chapter 10 - ethanol production from biomass. In: Tojo, S., Hirasawa, T. (Eds.), Research Approaches to Sustainable Biomass Systems. Academic Press, Boston, pp. 243–258. https://doi.org/10.1016/B978-0-12-404609-2.00010-6.

Jin, M., Slininger, P.J., Dien, B.S., Waghmode, S., Moser, B.R., Orjuela, A., Sousa Lda, C., Balan, V., 2015. Microbial lipid-based lignocellulosic biorefinery: feasibility and challenges. Trends Biotechnol. 33 (1), 43–54. https://doi.org/10.1016/j.tibtech.2014.11.005.

Jones, A.D., Boundy-Mills, K.L., Barla, G.F., Kumar, S., Ubanwa, B., Balan, V. 2019. Microbial lipid alternatives to plant lipids. in: Microbial lipid production: Methods and protocols, (Ed.) V. Balan, Springer New York. New York, NY, pp. 1-32. https://doi.org/10.1007/978-1-4939-9484-7_1.

Kieliszek, M., Kot, A.M., Bzducha-Wróbel, A., Błażejak, S., Gientka, I., Kurcz, A., 2017. Biotechnological use of Candida yeasts in the food industry: a review. Fungal Biol Rev. 31 (4), 185–198.

Ko, J.K., Lee, S.M., 2018. Advances in cellulosic conversion to fuels: engineering yeasts for cellulosic bioethanol and biodiesel production. Curr Opin Biotechnol. 50, 72–80. https://doi.org/10.1016/j.copbio.2017.11.007.

Kot, A.M., Błażejak, S., Kurcz, A., Bryś, J., Gientka, I., Bzducha-Wróbel, A., Maliszewska, M., Reczek, L., 2017. Effect of initial pH of medium with potato wastewater and glycerol on protein, lipid and carotenoid biosynthesis by *Rhodotorula glutinis*. Electron J Biotechnol. 27, 25–31. https://doi.org/10.1016/j.ejbt.2017.01.007.

Kumar, D., Singh, B., Korstad, J., 2017. Utilization of lignocellulosic biomass by oleaginous yeast and bacteria for production of biodiesel and renewable diesel. Renew. Sust. Energ Rev. 73, 654–671. https://doi.org/10.1016/j.rser.2017.01.022.

Liu, Z., Feist, A.M., Dragone, G., Mussatto, S.I., 2020. Lipid and carotenoid production from wheat straw hydrolysates by different oleaginous yeasts. J. Clean Prod. 249, 119308 https://doi.org/10.1016/j.jclepro.2019.119308.

Liu, Q., Lu, M., Jin, C., Hou, W., Zhao, L., Bao, J., 2022. Ultra-centrifugation force in adaptive evolution changes the cell structure of oleaginous yeast *Trichosporon cutaneum* into a favorable space for lipid accumulation. Biotechnol Bioeng. 119 (6), 1509–1521. https://doi.org/10.1002/bit.28060.

Liu, G., Zhang, Q., Li, H., Qureshi, A.S., Zhang, J., Bao, X., Bao, J., 2018. Dry biorefining maximizes the potentials of simultaneous saccharification and co-fermentation for cellulosic ethanol production. Biotechnol Bioeng. 115 (1), 60–69. https://doi.org/10.1002/bit.26444.

Moreno, J.M., Sanchez-Dmontero, J.M., Ballesteros, A., Sinisterra, J.V., 1991. Hydrolysis of nucleic acids in single-cell protein concentraions using immobilized benzonase. Appl. Biochem. Biotech. 31, 43–51. https://doi.org/10.1007/BF02922124.

Nunes, A.J.P., Sá, M.V.C., Browdy, C.L., Vazquez-Anon, M., 2014. Practical supplementation of shrimp and fish feeds with crystalline amino acids. Aquaculture 431, 20–27. https://doi.org/10.1016/j.aquaculture.2014.04.003.

Ritala, A., Hakkinen, S.T., Toivari, M., Wiebe, M.G., 2017. Single cell protein-state-of-the-art, industrial landscape and patents 2001–2016. Front Microbiol. 8, 2009. https://doi.org/10.1016/10.3389/fmicb.2017.02009.

Ruan, Z., Zanotti, M., Wang, X., Ducey, C., Liu, Y., 2012. Evaluation of lipid accumulation from lignocellulosic sugars by *Mortierella isabellina* for biodiesel production. Bioresour. Technol. 110, 198–205. https://doi.org/10.1016/j.biortech.2012.01.053.

Salazar-López, N.J., Barco-Mendoza, G.A., Zuñiga-Martínez, B.S., Domínguez-Avila, J.A., Robles-Sánchez, R.M., Ochoa, M.A.V., González-Aguilar, G.A. 2022. Single-cell protein production as a strategy to reincorporate food waste and agro by-products back into the processing chain. in: Bioengineering, Vol. 9. https://doi.org/10.3390/bioengineering9110623.

Sharif, M., Zafar, M.H., Aqib, A.I., Saeed, M., Farag, M.R., Alagawany, M., 2021. Single cell protein: Sources, mechanism of production, nutritional value and its uses in aquaculture nutrition. Aquaculture. 531, 735885 https://doi.org/10.1016/j.aquaculture.2020.735885.

Singh, S., Pandey, D., Saravanabhupathy, S., Daverey, A., Dutta, K., Arunachalam, K., 2022. Liquid wastes as a renewable feedstock for yeast biodiesel production: opportunities and challenges. Environ Res. 207, 112100 https://doi.org/10.1016/j.envres.2021.112100.

Sitepu, I.R., Garay, L.A., Sestric, R., Levin, D., Block, D.E., German, J.B., Boundy-Mills, K.L., 2014. Oleaginous yeasts for biodiesel: current and future trends in biology and production. Biotechnol Adv. 32 (7), 1336–1360. https://doi.org/10.1016/j.biotechadv.2014.08.003.

Sluiter, A., Hames, B., Scarlata, C., Sluiter, J., Templeton, D. 2012. Determination of structural carbohydrates and lignin in biomass national renewable. NREL/TP-510-42618. National Renewable Energy Laboratory, Golden, CO.

Sun, L., Shao, S., Bao, J., 2021. Microbial lipid fermentation of *Trichosporon cutaneum* in high saline water. Bioresour Bioprocess. 8 (1), 71. https://doi.org/10.1186/s40643-021-00424-z.

Thiviya, P., Gamage, A., Kapilan, R., Merah, O., Madhujith, T., 2022a. Production of single-cell protein from fruit peel wastes using palmyrah toddy yeast. Fermentation. 8 (8), 355. https://doi.org/10.3390/fermentation8080355.

Thiviya, P., Gamage, A., Kapilan, R., Merah, O., Madhujith, T., 2022b. Single cell protein production using different fruit waste: A review. Separations. 9 (7), 178. https://doi.org/10.3390/separations9070178.

Tian, Y., Li, J., Meng, J., Li, J., 2023. High-yield production of single-cell protein from starch processing wastewater using co-cultivation of yeasts. Bioresour Technol. 370, 128527 https://doi.org/10.1016/j.biortech.2022.128527.

Vasconcelos, B., Teixeira, J.C., Dragone, G., Teixeira, J.A., 2019. Oleaginous yeasts for sustainable lipid production—from biodiesel to surf boards, a wide range of "green" applications. Appl Microbiol Biotechnol. 103 (9), 3651–3667. https://doi.org/10.1007/s00253-019-09742-x.

Wada, O.Z., Vincent, A.S., Mackey, H.R., 2022. Single-cell protein production from purple non-sulphur bacteria-based wastewater treatment. Rev Environ Sci Bio-Technol. 21 (4), 931–956. https://doi.org/10.1007/s11157-022-09635-y.

Wang, J., Gao, Q., Zhang, H., Bao, J., 2016. Inhibitor degradation and lipid accumulation potentials of oleaginous yeast *Trichosporon cutaneum* using lignocellulose feedstock. Bioresour Technol. 218, 892–901. https://doi.org/10.1016/j.biortech.2016.06.130.

Wang, H., Hu, B., Liu, J., Qian, H., Xu, J., Zhang, W., 2020. Co-production of lipid, exopolysaccharide and single-cell protein by *Sporidiobolus pararoseus* under ammonia nitrogen-limited conditions. Bioprocess Biosyst Eng. 43 (8), 1403–1414. https://doi.org/10.1007/s00449-020-02335-3.

Xue, S.J., Chi, Z., Zhang, Y., Li, Y.F., Liu, G.L., Jiang, H., Hu, Z., Chi, Z.M., 2018. Fatty acids from oleaginous yeasts and yeast-like fungi and their potential applications. Crit Rev Biotechnol. 38 (7), 1049–1060. https://doi.org/10.1080/07388551.2018.1428167.

Yang, F., Jin, Z., Nawaz, M., Xiao, Y., Jiang, Y., Hu, J., Li, J., Gao, M.T., 2022. Oligosaccharides in straw hydrolysate could improve the production of single-cell protein with *Saccharomyces cerevisiae*. J Sci Food Agric. 102 (7), 2928–2936. https://doi.org/10.1002/jsfa.11633.

Zayed, G., Mostafa, N., 1992. Studies on the production and kinetic aspects of single cell protein from sugar cane bagasse saccharified by *Aspergillus Niger*. Biomass Bioenerg. 3 (5), 363–367. https://doi.org/10.1016/0961-9534(92)90009-F.

Zhang, Y., Bao, J., 2022. Tolerance of *Trichosporon cutaneum* to lignin derived phenolic aldehydes facilitate the cell growth and cellulosic lipid accumulation. J Biotechnol. 343, 32–37. https://doi.org/10.1016/j.jbiotec.2021.09.009.

Zhang, B., Khushik, F.A., Zhan, B., Bao, J., 2021a. Transformation of lignocellulose to starch-like carbohydrates by organic acid-catalyzed pretreatment and biological detoxification. Biotechnol Bioeng. 118 (10), 4105–4118. https://doi.org/10.1002/bit.27887.

Zhang, B., Zhan, B., Bao, J., 2021b. Reframing biorefinery processing chain of corn fiber for cellulosic ethanol production. Ind Crop Prod. 170, 113791 https://doi.org/10.1016/j.indcrop.2021.113791.

Zhang, X.Y., Zhao, G.J., 2022. Yeast cultivation for single-cell protein production using the carbohydrate hydrolysate of steam-exploded eucalyptus wood. Wood Res. 67 (4), 568–581. https://doi.org/10.37763/wr.1336-4561/67.4.568581.