

Valorizing Non-Food Carbon Sources: Discovery and Characterization of a *Cyberlindnera jadinii* for Single-Cell Protein SynthesisWei Zhong,[#] Chong Xie,[#] Kuan Chen, Nana Liu, Chengchao Zhu, Junze Liu, Huiqi Sun, Jingliang Xu, Lei Wang, Chuan Xia,^{*} and Yajie Wang^{*}Cite This: *ACS Sustain. Chem. Eng.* 2026, 14, 16145–16159

Read Online

ACCESS |



Metrics & More



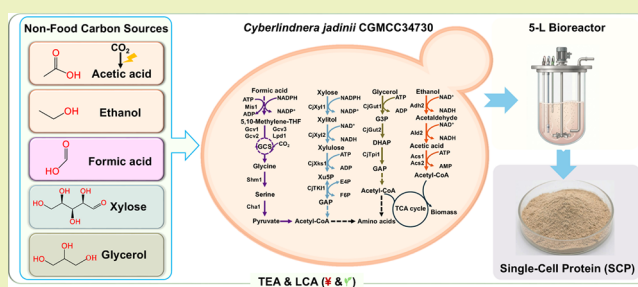
Article Recommendations



Supporting Information

ABSTRACT: Developing sustainable single-cell protein (SCP) from non-food feedstocks offers a promising strategy to address the escalating global demand for sustainable nutrition. However, the lack of industrially robust platforms capable of cost-efficient multi-substrate assimilation remains a key bottleneck. Here, we discover a *Cyberlindnera jadinii* strain, CGMCC34730, which can efficiently utilize diverse non-food carbon sources (e.g., acetate, ethanol, and xylose) for SCP production. Metabolic analysis reveals the assimilation mechanisms for these substrates, notably identifying the reductive glycine pathway as central to formic acid (FA) utilization. Scaled-up production in a 5-L bioreactor demonstrates excellent performance, with ethanol- and acetate-driven cultures reaching protein contents of 68.60 and 67.34% and volumetric productivities of 8.91 and 7.90 t m⁻³ y⁻¹, respectively. The resulting SCP surpasses soybean meal and approaches the quality of fish meal, exhibiting superior essential amino acid indices alongside elevated carbohydrate and B-vitamin contents. Furthermore, we validate a circular bioeconomy model by converting electrochemically synthesized ¹³C-labeled acetate into SCP. Techno-economic analysis confirms that acetate-based fermentation offers optimal cost-effectiveness for industrial deployment. In summary, this GRAS-certified platform establishes a highly efficient and economically viable route for the sustainable production of high-value SCP from non-food substrates.

KEYWORDS: *Cyberlindnera jadinii*, single-cell protein, non-food carbon sources, assimilation pathways, techno-economic analysis



1. INTRODUCTION

With the global population projected to reach 9.8 billion by 2050, the demand for food protein is expected to surge by 30–50%, reaching 265 million tonnes.^{1,2} Furthermore, animal protein demand is projected to reach approximately 600 million tonnes by 2025, with an additional increase of 300 million tonnes expected every decade.³ Addressing this structural deficit requires biotechnological innovations in sustainable biomanufacturing that extend beyond traditional agronomic paradigms.^{4,5} Single-cell protein (SCP), derived from diverse microorganisms, including yeasts,^{6–11} fungi,¹² algae,^{13,14} and bacteria,^{15–18} offers a nutritionally complete and resource-efficient alternative to conventional animal and plant proteins. SCP production utilizes various carbon sources, generally categorized as sugar-based substrates (e.g., glucose in *Fusarium venenatum*¹⁹) or non-sugar substrates. The sugar-based fermentation competes directly with the human food supply.¹ The non-sugar substrates include waste streams (e.g., *Yarrowia lipolytica* on biofuel waste,²⁰ *Kluyveromyces marxianus* on corn straw,⁶ *Cyberlindnera jadinii* on dihydroxyacetone,⁷ *Pichia pastoris* on methanol,^{10,11} *C. jadinii* on ethanol,²¹ *Acetobacterium* on acetate,¹⁸ and macroalgae²² and *Clostridium autoethanogenum* on inorganic carbon.¹⁷ Currently, non-sugar alternatives encounter challenges such as

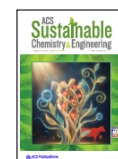
contamination risks (e.g., heavy metals and polycyclic aromatic hydrocarbons in biofuel waste²³), high costs associated with substrate pretreatment (increasing costs by 25–40% for corn straw²⁴), cost-intensive electrocatalytic acetate (HOAc) synthesis with *Acetobacterium*,¹⁸ and safety concerns regarding methanol handling and residue control.^{25,26} Furthermore, with respect to CO₂-derived SCP, macroalgae systems are limited by low photosynthetic efficiency (≤3%) and lower protein content (3–47%).¹⁴ Conversely, while *C. autoethanogenum* achieves higher protein yields (60–70%), it requires strict anaerobic conditions and H₂-dependent reducing power, which increases production costs by more than 50% compared with traditional sugar-based aerobic fermentation systems.²⁷ Overall, for anaerobic bacteria SCP production, strict anaerobic cultivation leads to excessive energy

Received: March 13, 2026

Revised: July 10, 2026

Accepted: July 13, 2026

Published: August 28, 2026



expenditure that contributes more than half of total production costs, while their biosafety profiles have not been fully verified.²⁷ Microalgal SCP manufacturing is constrained by technical and cost barriers such as low photosynthetic efficiency and fluctuating protein yields.¹⁴ Thus, the synergistic selection of robust microbial hosts and sustainable feedstocks is pivotal for advancing SCP biomanufacturing.

Among candidate platforms, the GRAS-certified yeast *C. jadinii* stands out as a premier chassis, distinguished by a protein content exceeding 50% (dry weight),^{28,29} an FAO/WHO-compliant amino acid profile,⁸ and a Crabtree-negative phenotype that inherently favors high-density biomass accumulation.³⁰ Beyond conventional sugar-centric paradigms,^{29,31} the strain's established ability to valorize diverse non-food substrates (e.g., dihydroxyacetone,⁷ xylose,³² and glycerol³³ suggests a latent capacity for processing simpler carbon architectures). This biological plasticity provides a compelling rationale to reposition *C. jadinii* as a specialized platform for C1–C2 feedstocks. By capitalizing on the low cost of HOAc³⁴ and the energy-dense, self-sterilizing nature of ethanol (EtOH),²⁸ this approach would establish a resilient, cost-effective framework for next-generation non-food biomanufacturing.

Herein, we establish *C. jadinii* CGMCC34730 as a robust platform for SCP production. To comprehensively assess this biomanufacturing strategy, this study first systematically characterizes the capacity of this yeast to efficiently utilize diverse non-food carbon sources (xylose, glycerol, HOAc, and EtOH). Subsequent metabolic analysis elucidates the underlying pathways, particularly highlighting the role of the reductive glycine (rGly) pathway in formic acid (FA) co-assimilation. Following this, we evaluate the scalable fermentation potential, the nutritional quality of the resultant SCP, and the feasibility of circular bioeconomy implementation using CO₂-electrosynthesized HOAc. Finally, techno-economic analysis (TEA) and life cycle assessment (LCA) confirm the industrial feasibility of the overall process. Collectively, these efforts provide a systematic framework for assessing the strain's capability in SCP production from non-food carbon sources and the associated techno-economic viability.

2. MATERIALS AND METHODS

2.1. Plasmids, Strains, and Genes

C. jadinii CGMCC34730 was employed as the host strain for strain construction, while *Escherichia coli* Trans1-T1 was utilized for plasmid construction. The details of all strains and plasmids used in this study can be found in Table S1. Enzymes involved in the assimilation of FA, EtOH, and HOAc—including glycine decarboxylase (*GCV2*), formate-tetrahydrofolate ligase (*ADE3*), mitochondrial C1-tetrahydrofolate synthase (*MIS1*), alcohol dehydrogenase (*ADH2*), aldehyde dehydrogenases (*ALD2*), and acetyl-CoA synthetases (*ACS1* and *ACS2*)—were identified through amino acid sequence alignment and annotated using KEGG and GO databases.³⁵ All gene sequences were obtained by sequencing the genome of *C. jadinii* CGMCC34730.

2.2. Reagents and Chemicals

Primers were synthesized by Sangon Biotech (China). PCR reactions were conducted using KOD plus Neo and KOD FX201 DNA polymerases (Toyobo, Japan). Restriction enzymes and ligases for plasmid construction were obtained from New England Biolabs (USA). The assembly of multiple DNA fragments for plasmid construction was performed using the ClonExpress MultiS One-Step Cloning Kit (Vazyme Biotech, China). Plasmid isolation and DNA purification were carried

out using the kits from Biomed Biotechnology Company Limited (China).

The ¹³C-FA (Sigma-Aldrich, 279404-1G) was purchased from Sigma-Aldrich. The NaH¹³CO₃ (Aldrich, S331496-1 g) was purchased from Aldrich. The mass spectrometry-grade ammonium HOAc, ammonium hydroxide, and acetonitrile were purchased from Sigma. Additional reagents, including analytical standards, were obtained from Aldrich. All vitamins were purchased from Sangon Biotech (Shanghai, China).

2.3. Medium and Growth Conditions

C. jadinii CGMCC34730 and its mutants were typically cultured in Yeast Extract Peptone Dextrose (YPD) medium (20 g/L peptone, 20 g/L glucose, and 10 g/L yeast extract) or Yeast Extract Peptone Xylose (YPX) medium. Solid media for *C. jadinii* CGMCC34730 were prepared by adding 15 g/L agar to the corresponding liquid medium. Growth on diverse carbon sources was evaluated in the Delft medium supplemented with different carbon sources (HOAc, FA, EtOH, glycerol, xylose, and glucose). The Delft consists of 7.5 g/L (NH₄)₂SO₄, 14.4 g/L KH₂PO₄, 2.1 g/L K₂HPO₄, 0.5 g/L MgSO₄·7H₂O, 1 mL/L vitamin solution (0.05 g/L biotin, 0.2 g/L p-amino benzoic acid, 1 g/L nicotinic acid, 1 g/L Calcium pantothenate, 1 g/L pyridoxine-HCl, 1 g/L thiamine-HCl, and 25 g/L myo-inositol at pH 6.5) and 2 mL/L trace metal solution (15.0 g/L EDTA sodium salt, 4.5 g/L ZnSO₄·7H₂O, 0.84 g/L MnCl₂·2H₂O, 0.3 g/L CoCl₂·6H₂O, 0.3 g/L CuSO₄·5H₂O, 0.4 g/L Na₂MoO₄·2H₂O, 4.5 g/L CaCl₂·2H₂O, 3 g/L FeSO₄·7H₂O, 1 g/L H₃BO₃, and 0.1 g/L Potassium Iodide at pH 4.0).

To evaluate the effect of FA on growth, the engineered strains were inoculated in Delft medium with FA (1 g/L and 2 g/L) and different carbon sources (20 g/L glucose, 10 g/L and 30 g/L EtOH, 20 g/L xylose, 20 g/L glycerol, and 3 g/L HOAc), and 200 mg/L of either G418 or Zeocin, followed by overnight incubation. This process was repeated three times. The cultures were washed twice with PBS (pH 7.2). The resulting cells were then inoculated in the same medium at an OD₆₀₀ of 0.05 and cultured at 30 °C for 3 to 4 days until reaching the stationary growth phase. Cultivation was performed in a 40-mL bottle at 30 °C under shaking.

The fed-batch fermentation was performed in a 5-L bioreactor (Bailun-5JG, Shanghai, China) with an initial working volume of 2 L of minimal medium. The medium composition was as follows: 14.4 g L⁻¹ KH₂PO₄, 0.5 g L⁻¹ MgSO₄·7H₂O, supplemented with trace metal and vitamin solutions. The cultivation was initiated by inoculating the bioreactor to an initial optical density at OD₆₀₀ of 1.5. The fermentation process was controlled at a constant temperature of 30 °C. The dissolved oxygen (DO) level was maintained at 30% saturation by cascading the agitation speed from an initial 250 rpm to a maximum of 950 rpm, with a constant aeration rate of 2 NL min⁻¹. The pH was automatically maintained at 5.0 through the addition of NH₃·H₂O (aqueous ammonia). During the fed-batch phase, a DO-stat feeding strategy was employed. Specifically, the feeding of 25% EtOH or HOAc was dynamically coupled to the DO signal; a sharp rise in DO indicated substrate depletion and automatically activated the feed pump.

2.4. Genetic Manipulation

The primers used in this study, synthesized by Sangon Biotech (China), are listed in Table S2. All gene sequences are in Table S3. Expression cassette components, such as promoters, genes, and terminators, were amplified using high-fidelity KOD FX201 DNA polymerase (Toyobo, Japan). Plasmid construction from two fragments was performed via digestion and ligation. Restriction enzymes and ligases were obtained from New England Biolabs (USA). Plasmid construction from multiple fragments was performed using the ClonExpress MultiS One-Step Cloning Kit (Vazyme Biotech, China). Plasmid isolation and DNA purification were carried out using kits from Biomed Biotechnology Company Limited (China). Gene knockout of *C. jadinii*

CGMCC34730 was performed using a homologous recombination method as described in previous studies.^{36,37} In this system, the commercial plasmid pPICZalpha A was used as the backbone to construct gene knockout vectors of *C. jadinii* CGMCC34730, namely pPICZalpha A-kan 3, pPICZalpha A-Zeocin, and pPICZalpha A-Hygromycin B. The kan/zeocin/Hygromycin B (HygB) resistance gene was driven by the GAP promoter and CYC1 terminator from *C. jadinii* CGMCC34730 (pGAP: kan/zeocin/HygB:Tyc1). Flanking homologous sequences of the target gene were inserted upstream and downstream of the antibiotic resistance expression cassette. The constructed plasmids were then used as templates for PCR amplification to generate fragments containing the upstream homologous arm, the resistance gene expression cassette, and the downstream homologous arm (Figure S1). The recombinant *C. jadinii* CGMCC34730 strains were obtained by electrotransforming (750 V, 800 Ω , 25 μ F) these fragments into *C. jadinii* CGMCC34730 cells using the lithium acetate method.³⁸

All modified genotypes were confirmed by colony PCR and sequencing. Further details on the preparation of competent cells and colony PCR are available in Supplementary Method 1.

2.5. ¹³C Metabolite Tracer Analysis

The methods for the ¹³C metabolite tracer analysis were adapted from a previous study.³⁹ For the analysis of ¹³C-labeled amino acids and metabolites within the central metabolic pathway, *C. jadinii* CGMCC34730 was first cultured in Delft with 10 g/L of unlabeled EtOH and FA as the carbon source for approximately 36 h, reaching the exponential growth phase. The cells were washed twice with PBS (pH 7.2) and transferred to Delft with 1 g/L ¹³C-labeled FA and 10 g/L ¹²C-EtOH at an OD₆₀₀ of 0.05 and incubated until the exponential growth phase. The resulting cell culture was washed with PBS and incubated in fresh Delft with 1 g/L ¹³C-labeled FA and 10 g/L ¹²C-EtOH until the exponential growth phase was reached again. This process was repeated for three generations. The 4th generation of cells incubated with ¹³C-FA and ¹²C-EtOH (OD₆₀₀ $\approx$ 0.53) was pelleted by centrifugation at 8000 $\times$ g and 4 $^{\circ}$ C for 3 min. The cells were then resuspended in a cold mixture of 300 μ L methanol at -20° C, 300 μ L chloroform at -20° C, and 250 μ L water at 4 $^{\circ}$ C. After vortexing, the supernatant was transferred to a pre-cooled 1.7 mL screw-cap tube, and 500 μ L of pre-cooled glass beads were added. Cell lysis was performed using a homogenizer (SCIENTZ-48LT, SCIENTZ Co., China) at 70 Hz, oscillating for 30 s with 15-second intervals, at 4 $^{\circ}$ C. The lysed cells were centrifuged at 10,000 $\times$ g and 4 $^{\circ}$ C for 10 min. The supernatant was flash-frozen with liquid nitrogen and lyophilized. Finally, the samples were reconstituted in 50 μ L of a 50:50 methanol-water mixture for analysis.

Analysis was conducted using an LC-MS system comprising an Agilent 1290 Infinity II UHPLC system in tandem with timsTOF Pro2 (Bruker). Extracted metabolites were dissolved in 100 μ L of 50% methanol for analysis. Chromatographic separation was achieved on an ACQUITY UPLC BEH Amide column (100 mm $\times$ 2.1 mm, 1.7 μ m). The mobile phase A consisted of 15 mM ammonium acetate and 0.3% NH₃·H₂O in water, and the mobile phase B consisted of 15 mM ammonium acetate and 0.3% NH₃·H₂O in 9:1 acetonitrile/water. The flow rate was 0.3 mL/min. The column was washed with 95% mobile phase B for 1 min, followed by a linear gradient to 50% over 8 min, held at 50% for 3 min, then a linear gradient back to 95% over 0.5 min, with a final 1.5 min at 95%. A 5 μ L sample was injected for each analysis.

For ¹³CO₂ experiments, the Delft medium with 10 g/L of unlabeled EtOH was supplemented with 1 g/L ¹²C-FA and 0.5 g/L NaH¹³CO₃. Other sample preparation and analysis methods were identical to the ¹³C-FA experiments. For all ¹³C-labeled experiments, the samples prepared with Delft medium supplemented with unlabeled formic acid or NaHCO₃ were used as negative controls. All final ¹³C incorporation data were corrected for natural isotopic abundance using the AccuCor2 algorithm of Bruker TASQ software.⁴⁰ The specific ¹³C

incorporation was calculated based on formula 1. The *n* represents the number of carbon atoms in the metabolite, and the *S_i* denotes the relative fraction of each isotopologue. The Fractional Contribution (FC) represents the average carbon labeling, which quantifies the mean percentage of isotopic labeling (e.g., ¹³C incorporation) per carbon atom in a metabolite.⁴⁰ Corrected Isotopologue Abundance represents the natural isotope abundance-corrected relative abundance (*s_i*) of a specific isotopologue (e.g., *M* + 1, *M* + 2), expressed as a percentage.⁴⁰ An example of labeling for glucose-6-phosphate is shown in Figure S2.

$$FC = \frac{\sum_{i=0}^n i s_i}{n} \quad (1)$$

2.6. Whole-Genome Sequencing and Data Analysis

Whole-genome sequencing of *C. jadinii* CGMCC 34730 was performed following culture in Delft with 1.5 g/L FA and 10 g/L EtOH. Genomic DNA extraction was performed using the NucleoBond HMW DNA kit (MN NucleoBond, Germany, 740160.20) to obtain high-quality genomic DNA from samples, with concentration and purity assessed via Qubit 4.0 (Thermo, Q33226) and integrity evaluated through 0.75% agarose gel electrophoresis. For library construction and sequencing, the whole genome DNA was randomly fragmented to an average size of 200–400 bp, followed by end-repair, 3'adenylation, adapter ligation, and PCR amplification. The purified library was qualified using the Qubit 4 fluorometer and its length assessed via 2% agarose gel electrophoresis before sequencing on the Illumina NovaSeq 6000 platform with 300 bp reads at Sangon Biotech (Shanghai, China). Post-sequencing, raw reads were filtered using Trimmomatic (v0.36) to remove adapters and low-quality reads, yielding clean reads. Genome assembly was conducted with SPAdes (v3.15),⁴¹ and gaps were filled using Gapfiller (v1.11).⁴² Gene prediction and annotation were performed using GeneMark (Version 1.10), with functional annotation based on protein-coding genes utilizing the euKaryotic Ortholog Groups (KOG) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.⁴³ The Comprehensive Antibiotic Resistance Database was queried to identify antibiotic resistance genes, while the Pathogen Host Interactions Database (PHI-base)⁴⁴ was used to locate target genes for drug interventions. Additionally, the Carbohydrate-Active enZYmes Database (CAZy)⁴⁵ was utilized to annotate genes encoding carbohydrate-active enzymes. Details of processes are provided in Supplementary Methods 2.

GenomeScope 2.0 was used to estimate genome size, repetitiveness, and heterozygosity by fitting a mixture of negative binomial distributions to the k-mer spectrum, whereas Smudgeplot (<https://github.com/KamilSJaron/smudgeplot>) was used to visualize genome structure and infer ploidy based on heterozygous k-mer pairs.⁴⁶ These tools take the k-mer spectrum as input, perform a nonlinear least-squares optimization to fit a mixture of negative binomial distributions, and output estimates for genome size, repetitiveness, and heterozygosity rates.⁴⁶ The analysis revealed a triploid genome, which was characterized by a plot with three major peaks and a low frequency of putative error k-mers (0.151%).

2.7. Transcriptome Profiling

Biological triplicates of strain CGMCC34730 were cultured in Delft medium containing 1 g/L FA and 10 g/L EtOH or only 10 g/L EtOH (control group). Cells were harvested during the logarithmic phase (OD₆₀₀ $\approx$ 0.53), flash-frozen, and subjected to total RNA extraction using TRIzol. High-quality RNA (OD_{260/280} = 1.8–2.2, OD_{260/280} $\geq$ 2.0, RQN $\geq$ 6.5, 28S:18S $\geq$ 1.0) was used for Illumina Stranded mRNA library preparation, including polyA selection, fragmentation, double-stranded cDNA synthesis, end repair, adapter ligation, and PCR amplification. Libraries were sequenced on the NovaSeq X Plus platform (2 $\times$ 150 bp). Raw reads were processed with Fastp, aligned

to the reference genome (GSA: CRA031663) via HISAT2, and assembled with StringTie.⁴⁷ Differentially expressed genes were identified using DESeq2 ($|\log_2FC| \geq 1$, $FDR < 0.05$) and DEGseq ($|\log_2FC| \geq 1$, $FDR < 0.001$), respectively, followed by GO/KEGG enrichment (Bonferroni-corrected $P < 0.05$) and alternative splicing analysis with rMATS.⁴⁸ Details of processes are provided in Supplementary Methods 3.

2.8. Hierarchical Clustering Tree Construction

To elucidate the genetic diversity and broad genotype-phenotype relationships between *C. jadinii* CGMCC 34730 and wild strains, a hierarchical clustering tree with average linkage was constructed based on biallelic SNPs. A total of 451,302 biallelic segregating sites identified across the 20 *C. jadinii* strains were utilized to infer their phylogenetic relationships.⁴⁹ Inter-strain pairwise identity-by-state (IBS) metrics were computed employing the SNPRelate package. A hierarchical clustering algorithm was implemented on the resultant distance matrix through the pvclust package. To evaluate cluster stability, bootstrap validation with 1000 resampling iterations was conducted using pvclust, generating approximately unbiased (AU) P-values to quantify node support probabilities across the dendrogram. Diploid (2n) strains were characterized by a 1:1 SNP ratio, whereas triploid (3n) strains showed a 1:2 SNP ratio. Aneuploidy was identified when these expected ratios were disrupted, and this was further validated by changes in coverage depth, which indicate alterations in chromosome number. Strains maintaining consistent SNP ratios along with proportional coverage were considered euploid, while those exhibiting noticeable deviations were classified as aneuploid.⁴⁹

2.9. Techno-Economic Analysis

Techno-economic analysis (TEA) is a systematic methodology employed to evaluate the economic viability and technical feasibility of single-cell protein (SCP) production using EtOH and HOAc as feedstocks. The analysis was conducted based on the annuity method, which is particularly suited for assessing the long-term economic sustainability of bioprocesses by annualizing capital costs over the project's lifespan. Key technical assumptions, scale-up parameters, and comprehensive mass and energy balance data used in the modeling are detailed in Tables S4–S8. The analysis primarily focused on the inside battery limit (ISBL) costs, which encompass direct capital investments for process equipment, installation, and piping within the production facility. To account for the full project scope, outside battery limit (OSBL) costs, including infrastructure, warehousing, and site development, were also incorporated into the total capital expenditure (CapEx). Furthermore, operational expenditures (OpEx), such as raw materials, utilities, and labor, were estimated based on the process simulations and current market prices. This systematic TEA framework allows for the identification of key cost drivers and the assessment of the potential for cost reduction through process optimization and economies of scale. The Equipment cost is calculated using Formula 2.

$$\text{Equipment cost} = \text{ref cost}^* \left(\frac{\text{scale}}{\text{ref scale}} \right)^{\text{scaling factor}} \quad (2)$$

The biorefinery is projected to have an annual SCP production capacity of 37100 tons, with this capacity determined based on comprehensive techno-economic modeling that accounts for equipment sizing, raw material availability, and economic constraints. The capital expenditure (CapEx) and operational expenditure (OPEX) estimates were derived from published literature and market prices at the time of writing (Tables S7 and S8). Equipment costs were scaled according to the implementation size using a power law relationship relative to the base price.⁵⁰ All costs were adjusted to 2025 CNY to account for inflation and mitigate the impact of currency exchange rate fluctuations.

2.10. Life Cycle Assessment

The goal of this life cycle assessment (LCA) was to quantify and compare the environmental impacts associated with the production of single-cell protein (SCP) using EtOH or HOAc as feedstocks. The study was conducted in accordance with the ISO 14044 standard, employing a cradle-to-gate system boundary. This boundary encompasses all stages from raw material extraction (including the production of the carbon sources) to the synthesis of the final SCP biomass at the factory gate. The functional unit was defined as 1 kilogram (kg) of dry SCP biomass produced. The environmental impact assessment was performed using the CML 2002 baseline methodology,⁵¹ which calculates a set of standardized environmental impact indicators. A comprehensive life cycle inventory (LCI) detailing the material and energy flows for the production of 1 kg of SCP is provided in Figure 5c. The analysis specifically focused on the global warming potential (GWP), reported in kg of CO₂-equivalent (kg CO₂-eq), among other impact categories. For scenarios 2 and 4, the cut-off approach was applied, assigning zero environmental burden to the waste feedstock entering the system boundary. Regarding biogenic carbon, the carbon contained in the final SCP biomass was treated as neutral; it was not deducted from the GWP calculation, adhering to the ISO 14067 standard for short-lived food and feed products. Background data for upstream processes (e.g., electricity generation, chemical production) were sourced from the Ecoinvent v3.9 and the Hydregrdatabse. All LCA modeling, calculations, and impact assessments were carried out using the openLCA software version 2.2.

2.11. Cellular Composition Analysis

The harvested microbial cells for amino acid analysis were freeze-dried using a vacuum freeze-dryer. The lyophilized yeast samples were subjected to acid hydrolysis prior to amino acid quantification using an amino acid analyzer. Essential amino acid index (EAAI) was used to evaluate the nutritional quality of proteins according to Formula 3. Notably, tryptophan content was determined following alkaline hydrolysis of the samples. The carbohydrate content was quantified using the sulfuric acid-anthrone method.⁵² The obtained samples were analyzed for crude protein content (GB/T 6432-2018), crude fiber (GB/T 6434-2022), B vitamins (GB 5009.84-2016, GB 5009.85-2016, GB 5009.210-2013, GB 5009.154-2013 and GB 5009.285-2022), and crude fat (GB/T 14772-2008).

$$\text{EAAI} = \sqrt[n]{\frac{a1}{A1} \times \frac{a2}{A2} \times \dots \times \frac{an}{An}} \quad (3)$$

where an and An are the contents of EAAs in the sample and reference protein, respectively, and $n = 9$. Amino acid composition is expressed as grams per 100 g of dry protein. The EAAI was calculated using the essential amino acid reference pattern for adults recommended by the FAO (2013).

2.12. Crude Protein and Amino Acid Test

Crude protein content was determined via the Kjeldahl method.⁵³

(1) Sample preparation: 0.2 g of freeze-dried cells was weighed and transferred into a digestion tube. A mixed catalyst (3.6 g potassium sulfate and 0.4 g copper sulfate) and 10 mL concentrated sulfuric acid were subsequently added. A blank control was prepared simultaneously. (2) Sample digestion: All sealed digestion tubes were placed on a graphite digester for gradient temperature digestion. Small funnels were fitted for digestion when fewer than 10 samples were tested. For 10 to 20 samples, a waste extraction hood was installed, and empty digestion tubes filled with 10 mL concentrated sulfuric acid were supplemented to standardize the total tube number to 20. (3) Kjeldahl determination: Distillation was initiated after the digestion tubes fully cooled. Reagent availability was checked beforehand, and cooling water

as well as instrument power were turned on. The device was preheated through two rounds of empty-tube distillation, with 30 mL dilution water, 20 mL boric acid and 10 mL alkali solution added for a 5-minute distillation duration. The blank sample was assayed first to correct systematic and reagent-derived errors, followed by the detection of experimental samples, wherein 40 mL of alkali solution was applied while other parameters remained unchanged. Consecutive sample testing was carried out without inter-tube cleaning. The distillate was promptly titrated with 0.1 mol/L standard hydrochloric acid solution until the pale gray-red titration endpoint appeared.

Amino acids were quantified using a post-column derivatization method.⁵⁴

(1) Sample acidification: An aliquot of m mg sample was weighed, and 10 mL of 1:1 analytical grade hydrochloric acid (approximately 6 mol/L) was slowly added. The mixture was purged with nitrogen for 15 min using a nitrogen evaporator, and the tube was then sealed. (2) High-temperature hydrolysis: The sealed sample tubes were placed in an oven and hydrolyzed at 110 °C for 24 h, followed by cooling and uncapping. (3) Deacidification: The hydrolyzed sample was diluted to a constant volume of 100 mL. Exactly 2 mL of the diluted solution was transferred and dried completely at 60 °C using a rotary evaporator for deacidification. (4) Instrumental analysis: Firstly, 2.0 mL of 0.02 mol/L hydrochloric acid was accurately added to the dried residue, mixed thoroughly by vortexing, and filtered through a 0.22 μ m aqueous membrane prior to injection. Amino acids were separated on a cation-exchange column and subjected to post-column derivatization with ninhydrin; the derived products were determined by a visible light detector to quantify amino acid concentrations. The analytical parameters were set as follows: separation column size, 4.6 mm ID $\times$ 60 mm; packing resin, Hitachi-specific cation exchange resin; limit of detection, 2.5 pmol; detection wavelengths, 570 nm and 440 nm; column temperature, programmed from 55 to 65 and then to 77 °C; reaction temperature, 135 °C; injection volume, 20 μ L; detection instrument, Hitachi LA8080. The amino acid content was calculated by the Formula 4:

$$\text{Content (\%)} = \frac{c^*v^*}{m} 100 \quad (4)$$

where c is the determined amino acid concentration of the test solution, v refers to the fixed volume of the hydrolyzed sample solution, and m is the weighing mass of the raw sample.

2.13. Intracellular NADH Assay

NADH quantification in *C. jadinii* CGMCC34730 was performed on biological triplicates pre-cultured in Delft medium containing 20 g/L carbon source (xylose, glucose, EtOH, HOAc, or glycerol) supplemented with 1 g/L FA. Cells were harvested during the early and late exponential phases, with cultures in unsupplemented Delft medium serving as controls. Cell pellets were resuspended in 600 μ L sterile ddH₂O and mechanically lysed by adding 250 μ L ice-cold glass beads ($\varnothing$ 0.5 mm), followed by homogenization using a SCIENTZ-48LT homogenizer (SCIENTZ Co., China) under the following conditions: 70 Hz oscillation frequency, 30-second cycles with 15-second intervals, at 4 °C. NADH levels in lysates were quantified using the Amplitude NADH Assay Kit (AAT Bioquest, USA) following manufacturer protocols and established methods.⁵⁵ This fluorometric assay employs an enzymatic cycling reaction for specific NADH detection and signal amplification. Briefly, lysates and NADH standards (serial dilutions; standard curve in Figure S3) were loaded (50 μ L/well) into black 96-well plates; reaction mixtures were incubated at room temperature for 60 min (light-protected), and fluorescence was measured kinetically (30 min) using a BioTek Synergy H1 microplate reader (Agilent, USA) at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 540/590$ nm.

3. RESULTS AND DISCUSSION

3.1. *C. jadinii* CGMCC34730 Isolation and Carbon Source Characterization

In this study, a *C. jadinii* strain was isolated from soil and deposited at the China General Microbiological Culture Collection Center (CGMCC) under accession number CGMCC34730. Analysis of ploidy and genome structure using GenomeScope 2.0 and Smudgeplot⁴⁶ revealed that *C. jadinii* CGMCC34730 possesses a triploid genome (Figure 1a). Hierarchical clustering based on biallelic SNPs resolved the *C. jadinii* population into three evolutionary clades (Classes I-III, Figure 1b). Notably, *C. jadinii* CGMCC34730 clustered within Class I, a genetic cluster containing both diploid and triploid strains, indicative of substantial ploidy variation, whereas Class III appeared strictly diploid. This polyploid state coincided with robust phenotypic performance: the triploid *C. jadinii* CGMCC34730 exhibited a 22% increase in cell density ($\text{OD}_{600} = 52.5$) compared to the diploid reference strain ATCC 15239 ($\text{OD}_{600} = 43.1$) in YPX medium (Figure S4a), underscoring its potentially superior capacity for industrial biomass accumulation.

To characterize the growth phenotype of *C. jadinii* CGMCC34730, we cultured the strain in Delft medium supplemented with various carbon sources. It exhibited broad metabolic versatility, achieving robust growth on EtOH ($\text{OD}_{600} 43.2 \pm 1.7$), HOAc (37.9 ± 0.6), glycerol (39.1 ± 3.1), xylose (33.6 ± 0.5), and glucose (33.6 ± 1.3) (Figure 1c). Notably, the maximum optical densities on EtOH and HOAc were significantly greater than those on the other substrates. These traits highlight the potential of *C. jadinii* for fermentation processes utilizing unconventional feedstocks. Furthermore, FA exhibited a stimulatory effect on the growth of *C. jadinii* CGMCC34730 in Delft medium containing either xylose or EtOH compared to the control conditions (Figure 1d,e). Specifically, the addition of 2 g/L FA enhanced the maximum OD_{600} by 30% compared to that of cultures grown on 20 g/L xylose alone (Figure 1d). Similarly, adding 1-2 g/L FA increased the maximum OD_{600} by 10 and 30% relative to cultures grown on 10 or 30 g/L EtOH alone, respectively (Figure 1d). This phenomenon was not observed in other media (Figure S4b-d). A plausible explanation lies in the dynamic regulation of NADH during EtOH and xylose assimilation.⁵⁶⁻⁵⁸ FA co-utilization reduces NADH accumulation in during xylose or ethanol assimilation *C. jadinii* CGMCC34730 (Figure S3). This suggests that the reducing equivalents generated during substrate assimilation may support FA assimilation and CO₂ fixation. These findings suggest that *C. jadinii* CGMCC34730 possesses the potential for FA utilization when co-cultured with specific carbon sources.

3.2. Metabolic Pathways for the Utilization of Various Carbon Sources

The *C. jadinii* CGMCC34730 strain exhibits a remarkable ability to utilize a wide range of non-food carbon sources. This metabolic versatility is attributed to the presence of specific, functional catabolic pathways within its cellular machinery. To date, key pathways have been identified for the assimilation of xylose (via the xylose reductase and xylitol dehydrogenase pathway),⁵⁸ EtOH (via alcohol dehydrogenase and aldehyde dehydrogenase),²⁸ HOAc (via acetyl-CoA synthetase),²⁸ and glycerol (via glycerol kinase and glycerol-3-phosphate dehydrogenase)⁵⁹ (Figure 2). Through comprehensive protein sequence alignment, we have successfully identified and

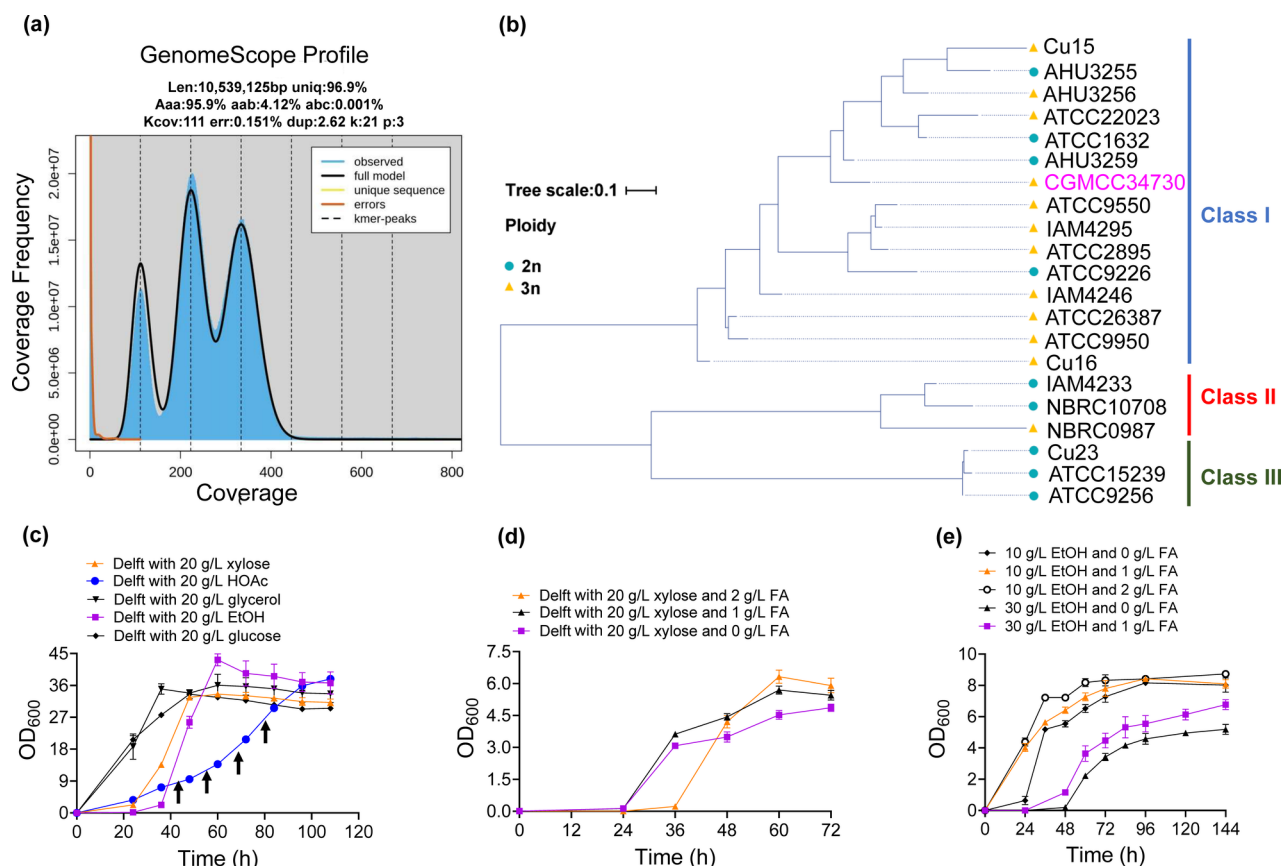


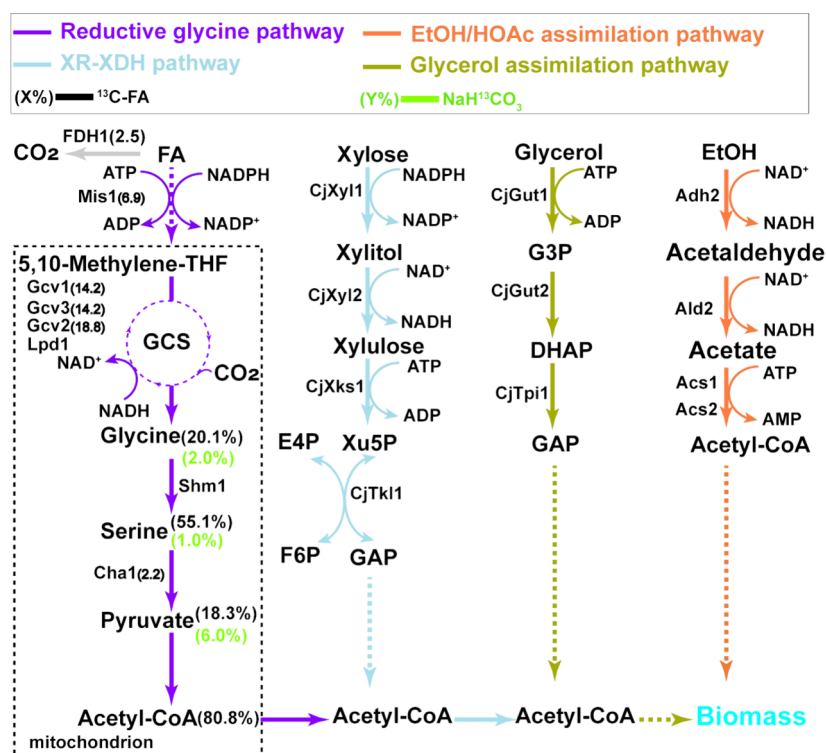
Figure 1. Ploidy analysis and carbon source characterization of isolated *C. jadinii* CGMCC34730. (a) GenomeScope k-mer analysis confirming the ploidy of *C. jadinii* CGMCC34730. k-mer frequency distribution displays three distinct major peaks with a low estimated sequencing error rate ($\approx 0.151\%$), consistent with a triploid genome. (b) Hierarchical clustering of 21 *C. jadinii* strains including CGMCC34730 based on biallelic SNPs. Symbols indicate ploidy: diploid strains (light blue circles) and the triploid CGMCC34730 (yellow triangle). Strains cluster into three groups: Class I (blue), Class II (red), and Class III (dark green). Scale bar represents genetic divergence; branches are statistically supported (AU P -values > 0.95). (c) Growth profile of *C. jadinii* CGMCC34730 on xylose, HOAc, glycerol, EtOH, and glucose. Arrows denote pulsed HOAc feeding (4.3 g L^{-1} per pulse; 20 g L^{-1} cumulative concentration). (d, e) Effect of FA on xylose-dependent (d) and EtOH-dependent (e) growth. Unless otherwise noted, *C. jadinii* CGMCC34730 was cultivated in Delft medium at 30°C and 180 rpm. Data represent mean $\pm$ s.d. ($n = 3$ biologically independent samples).

annotated the homologous enzymes responsible for these metabolic functions in the CGMCC34730 genome, as detailed in Table S3.

While FA oxidase exists in *C. jadinii* species,⁶⁰ the FA assimilation pathways involved remain uncharacterized. Here, we observed that co-feeding FA with either xylose or EtOH significantly enhanced the growth of *C. jadinii* CGMCC34730 (Figure 1d,e), suggesting a metabolic capability not previously reported for this species. To elucidate the underlying pathways, we conducted ^{13}C -labelling experiments using $1 \text{ g/L } ^{13}\text{C}$ -FA in Delft medium with 10 g/L EtOH . Isotopic analysis revealed broad carbon incorporation, with enrichment exceeding 20% in nine amino acids (glycine 20.1%, serine 55.1%, etc.) and more than 18% in fourteen central metabolites (acetyl-CoA 80.8%, pyruvate 18.3%, etc.), including key intermediates in the TCA cycle (Figures 3a,b and S5a,b). The low glycine enrichment (20.1%) versus serine (55.1%) may result from mitochondrial glycine export to the cytosol and its conversion to serine.⁶¹ These patterns confirm the active flux of FA-derived carbon into both amino acid biosynthesis and core energy metabolism. Furthermore, transcriptomic profiling

revealed significant upregulation of key genes involved in FA metabolism (e.g., *ENO*, *GCV1-3*, *ADE3*, *MIS1*, and *GPM2*) during FA co-utilization (Figure S6), indicating a reinforcement of central carbon metabolism.

Based on ^{13}C -labelling and transcriptomic data, we propose two potential FA assimilation pathways in *C. jadinii* CGMCC34730: the mitochondrial reductive glycine (rGly) pathway and the cytoplasmic glyoxylate-serine cycle (GSC) (Figures 2 and S7). We next integrated $^{13}\text{CO}_2$ -labelling, transcriptomic, and gene knockout analyses to distinguish between these mechanisms. Experiments using $\text{NaH}^{13}\text{CO}_3/^{12}\text{C}$ -EtOH in Delft revealed ^{13}C incorporation into glycine (2.0%), serine (1.0%), and TCA cycle metabolites (Figures 3c,d and S5c,d), suggesting rGly pathway activity. This finding was supported by transcriptomic profiling, which showed substantial upregulation of rGly genes—specifically *GCV1*, *GCV2*, and *GCV3* (14.2- to 18.8-fold) and *CHA1* (2.2-fold)—under FA conditions (Figure S6). Furthermore, strains with a partial deletion of *GCV2* or a complete deletion of *GCV1*, which encode components of the glycine cleavage system, exhibited severe growth delays during the co-utilization of FA with EtOH or xylose



The percentage beside the metabolites indicates the proportion of ^{13}C -labeled species for metabolite.

The number beside the gene indicates the fold of increased transcript abundance for gene.

Figure 2. Metabolic pathways for the assimilation of FA, HOAc, EtOH, glycerol, and xylose in *C. jadinii* CGMCC34730. Colored arrows delineate specific metabolic routes: reductive glycine pathway (purple); XR-XDH xylose assimilation pathway (blue); EtOH and HOAc assimilation pathways (orange); and glycerol assimilation pathways (olive green). Percentages denote fractional ^{13}C -labeling enrichment of metabolites. Numbers adjacent to gene symbols indicate the fold-change in transcript abundance in response to FA. Metabolite abbreviations: G3P, glycerol 3-phosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; Xu5P, xylulose 5-phosphate. Enzyme abbreviations: *Fdh1*, formate dehydrogenase; *CjXyl1*, xylose reductase; *CjXyl2*, xylitol dehydrogenase; *CjXks1*, xylulose kinase; *Tpi1*, triose phosphate isomerase; *CjTk1*, transketolase; *CjGut1*, glycerol kinase; *CjGut2*, mitochondrial glycerol-3-phosphate dehydrogenase; *Adh2*, alcohol dehydrogenase; *Ald2*, cytoplasmic aldehyde dehydrogenase; *Acs1/Acs2*, acetyl-CoA synthetase; *Cha1*, catabolic L-serine (L-threonine) deaminase; *Shm1*, mitochondrial serine hydroxymethyltransferase; *Mis1*, mitochondrial C1-tetrahydrofolate synthase; and *Gcv1/Gcv2/Gcv3*, mitochondrial glycine decarboxylase complex.

(Figure S8a–f). This phenotype may be attributed to the function of the rGly pathway in balancing cellular cofactor, specifically by consuming excess NADH generated from EtOH and xylose metabolism.^{56–58} Results confirmed that the ΔGCV strain accumulated substantial amounts of NADH when grown on EtOH or xylose (Figure S3). In contrast, the strain with a partial deletion of *ADE3* (*CJ- ΔADE3*) exhibited no growth retardation or NADH accumulation (Figure S3), and showed growth kinetics comparable to the wild-type control (CGMCC34730). The low targeted editing efficiency in this strain may arise from three factors: it exhibits strong non-homologous end joining (NHEJ) yet weak homologous recombination (HR), wherein NHEJ preferentially repairs DNA double-strand breaks and inhibits precise HR-mediated editing;⁶² as polyploids bear multiple target gene alleles on homologous chromosomes, editing tools cannot knock out all alleles simultaneously and thus produce chimeric transformants with partial gene disruption;⁶³ additionally, abundant wild-type alleles compete for limited homologous repair templates, drastically diminishing uniform recombination across all gene copies.⁶³ Collectively, the ^{13}C -labeling patterns consistent with rGly pathway activity, robust transcriptional expression of pathway genes, and growth phenotypic data obtained from rGly

pathway gene knockout strains demonstrate that the rGly pathway plays a critical role in NADH regulation during the co-utilization of FA with xylose or EtOH. More broadly, these findings highlight potential evolutionary constraint in eukaryotic FA assimilation. Among yeasts, *S. cerevisiae* is limited to the less efficient GSC pathway,³⁹ whereas *P. pastoris* has evolved an oxygen-tolerant rGly pathway,⁶⁴ indicating restricted evolutionary adaptability. Bacterial systems, however, have overcome these constraints more effectively: *Cupriavidus necator* achieves a biomass yield of 2.9 gCDW/mol-FA via its rGly pathway,⁶⁵ and *Vibrio natriegens* can grow autotrophically on FA alone using a serine cycle.⁶⁶ These comparisons highlight that eukaryotes, despite possessing rGly or GSC pathways, remain fundamentally constrained in converting FA to biomass at high efficiency, likely due to suboptimal redox coupling and compartmentalization challenges absent in prokaryotes.

3.3. *C. jadinii* CGMCC34730 as a Versatile Platform for SCP Production from Non-Food Carbon Sources

To assess the potential of *C. jadinii* CGMCC34730 as a platform for SCP production across different scenarios, we assessed its capacity to convert C_2 – C_5 substrates into SCP in shake flask fermentation. The results indicated that cells utilizing C_2

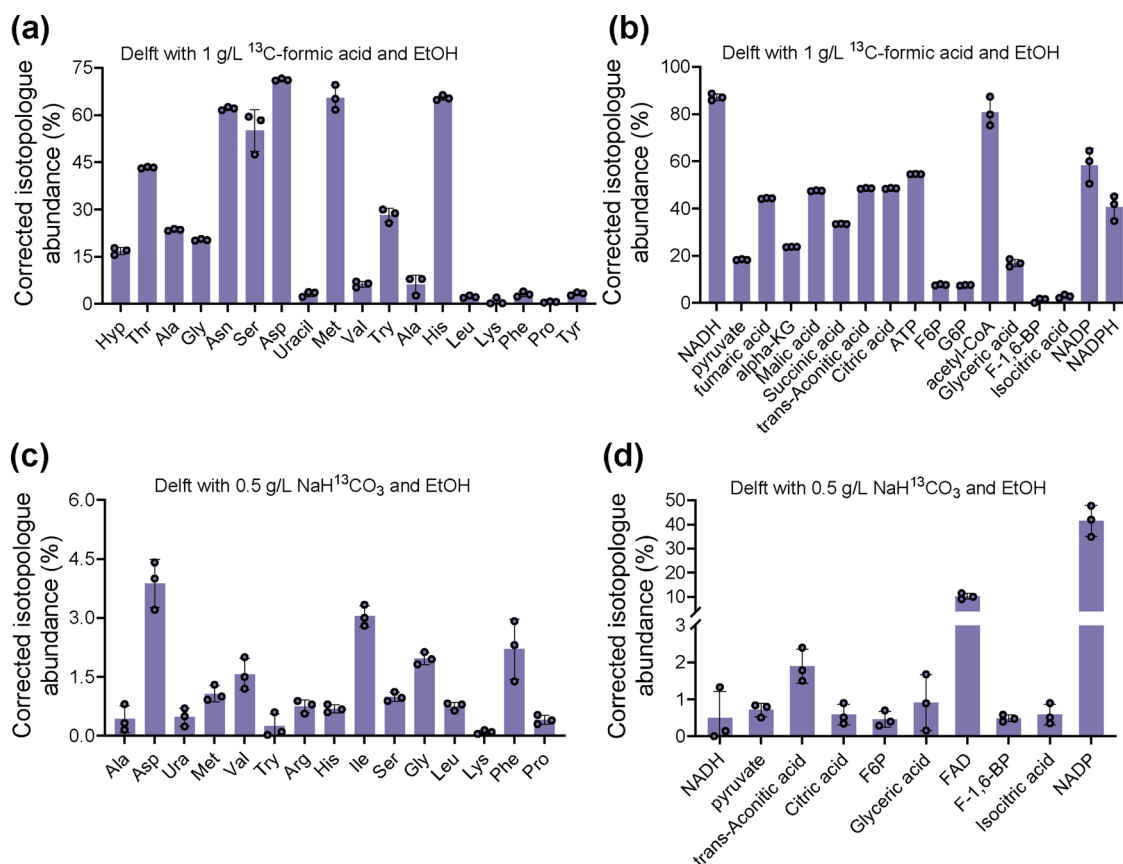


Figure 3. ^{13}C -isotopic analysis of the metabolites in CGMCC34730 during FA fermentation. (a) ^{13}C -FA labeled amino acids. (b) ^{13}C -FA labeled central metabolites. (c) $\text{NaH}^{13}\text{CO}_3$ labeled amino acids. (d) $\text{NaH}^{13}\text{CO}_3$ labeled central metabolites. For ^{13}C labeling experiments, *C. jadinii* was pre-cultured for three generations in Delft medium with 10 g/L ^{12}C -EtOH with either 1 g/L ^{13}C -FA (a,b) or 0.5 g/L $\text{NaH}^{13}\text{CO}_3$ (c,d) before being subcultured in the same medium at 30 °C and 180 r.p.m. until middle log phase for analysis. The full names of abbreviations are summarized in Table S13. All data represent the mean $\pm$ s.d. ($n = 3$ biologically independent samples). Metabolite abbreviations: *Met*, methionine; *Pro*, proline; *Thr*, threonine; *Ala*, alanine; *Gly*, glycine; *Asp*, aspartic acid; *Ser*, serine; *Try*, tryptophan; *Glu*, glutamic acid; *Asn*, asparagine; *Phe*, phenylalanine; *Lys*, lysine; *Arg*, arginine; *Orn*, ornithine; *Cit*, citrulline; *Val*, valine; *Ile*, isoleucine; *Pyr*, pyruvate; α -KG, α -ketoglutaric acid; *ATP*, adenosine triphosphate; *F6P*, fructose 6-phosphate; *R5P*, ribose 5-phosphate; *FAD*, flavin adenine dinucleotide; *2-PG*, 2-phosphoglycerate; *3-PG*, 3-phospho-D-glycerate; *F-1,6-BP*, fructose 1,6-bisphosphate; *PEP*, phosphoenolpyruvic acid; *G6P*, glucose-6-phosphate; *NADH*, reduced nicotinamide adenine dinucleotide; *NADP*, nicotinamide adenine dinucleotide phosphate; and *NADPH*, Nicotinamide adenine dinucleotide phosphate (reduced form).

substrates, specifically EtOH and HOAc, exhibited superior protein contents, reaching 62.6 and 60.9%, respectively (Figures 4a and S9). While supplementation with FA as an auxiliary carbon source increased SCP production in shake flasks (yielding increases of 10.9 and 4.8% for EtOH and HOAc, respectively), this enhancement was not observed during scale-up fermentation (data not shown). The absence of SCP accumulation and biomass growth during FA-EtOH co-fermentation in a 5-L bioreactor likely stems from suboptimal process conditions, primarily oxygen transfer limitations due to the high respiratory demands of SCP biosynthesis and substrate oxidation,⁶⁷ coupled with inhibitory effects from elevated EtOH concentrations that may suppress dehydrogenase activity and disrupt FA utilization.⁶⁸ Consequently, subsequent scale-up fermentations focused exclusively on EtOH and HOAc as the sole carbon sources.

Fed-batch fermentations were conducted using $\text{NH}_3 \cdot \text{H}_2\text{O}$ as the nitrogen source, starting with an initial OD_{600} of approximately 1.5. *C. jadinii* CGMCC34730 demonstrated continuous growth over 28 or 27 h. As detailed in Table S9 and Figure 4b,c,

the resulting biomass concentrations of WL01 (EtOH) and WL02 (HOAc) were 45.45 ± 0.35 and 39.60 ± 0.42 g DCW L^{-1} , respectively, with protein contents of 68.60 ± 0.97 and $67.34 \pm 0.07\%$. The corresponding biomass yields were 0.74 ± 0.01 g DCW g^{-1} EtOH and 0.44 ± 0.01 g DCW g^{-1} HOAc. Notably, the calculated SCP productivity reached 8.91 ± 0.06 and 7.90 ± 0.09 t $\text{m}^{-3} \text{y}^{-1}$, respectively, substantially exceeding the typical soybean yield of 1.4×10^{-4} t $\text{m}^{-3} \cdot \text{y}^{-1}$.⁶⁹ These findings confirm the viability of scaled-up SCP production and highlight the practical potential of this system.⁶⁹

To assess the potential of WL01 and WL02 as microbial protein sources for feed formulations, their amino acid compositions were compared with those of soybean meal and fishmeal.⁶ Both WL01 and WL02 presented well-balanced amino acid profiles (Figure S10). The total amino acid contents of WL01 (52.2%) and WL02 (45.9%) exceeded that of soybean meal (44.2%). This study observed a significant discrepancy (more than 16%) between crude protein (Kjeldahl method) and total amino acid content, primarily attributable to the inclusion of non-protein nitrogen (NPN) in Kjeldahl measurements.

As documented by previous studies,⁵³ Kjeldahl nitrogen incorporates NPN from nucleic acids (RNA/DNA), chitin, and metabolic intermediates. Furthermore, the total essential amino acid (EAA) content of WL01 (24.0%) was comparable to that of fishmeal (24.3%), while WL02's content (18.8%) was slightly lower; however, both are greater than that of soybean meal (15.8%). A radar plot visually highlights the superior amino acid balance of WL01 and WL02 relative to that of soybean meal, particularly with respect to branched-chain amino acids (Figure 4d). Additionally, the essential amino acid index (EAAI) values for WL01 and WL02 were 0.97 and 0.75, respectively (Table S10), which are superior to that of soybean meal (0.70). Cellular composition analysis further indicated that WL01 and WL02 presented relatively higher carbohydrate and B-vitamin contents, reinforcing their promise as SCP resources (Table S11). Comparative analysis demonstrated favorable protein content, biomass, and productivity for *C. jadinii* CGMCC34730 relative to established SCP-producing strains (Table 1). Collectively, these results support the potential of WL01 and WL02 as competitive protein sources for feed formulations.

In addition, we present a proof-of-concept for SCP production that utilizes CO₂ as the sole carbon source. This process utilizes abundant and readily available HOAc derived from CO₂ via electrolysis.⁷⁰ The growth profile of *C. jadinii* CGMCC34730 cultured on the HOAc-containing electrolyte was comparable to that on commercial HOAc (Figure S11). To verify that CO₂ serves as the sole carbon source for biomass

conversion, *C. jadinii* CGMCC34730 was cultivated using electrolysis-derived ¹³C-labelled HOAc (¹³CH₃¹³COOH) as the exclusive carbon substrate.⁷⁰ Analysis of the harvested, lyophilized biomass via Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS) revealed ¹³C_{TOC} accounted for 48.8% of the dry biomass (Table S12). This value aligns with the theoretical TOC of yeast, confirming that the biomass carbon originates primarily from CO₂-derived HOAc. These results demonstrate the technical feasibility of coupling *C. jadinii* CGMCC34730 fermentation with electrocatalysis for SCP production from CO₂.

3.4. Economic Feasibility Analysis and Life Cycle Assessment

We next performed a comprehensive evaluation integrating techno-economic analysis (TEA) and life cycle assessment (LCA), a combined framework now recognized as essential for determining the overall viability of emerging bioprocesses. To assess the economic and environmental viability of SCP production from C2 substrates, we modelled a large-scale process (Figure 5a) across four baseline scenarios: commercial EtOH (S1), wastewater EtOH (S2), commercial HOAc (S3), and wastewater HOAc (S4). The model projects an annual capacity of over 37,100 tons, representing approximately 2.9% of the global SCP market and 7.03% of the yeast-derived market.⁷¹ Driven by the advantages of waste-derived feedstocks and economies of scale, S4 achieved the lowest production cost at 3059 CNY/t SCP, substantially below the previously reported values

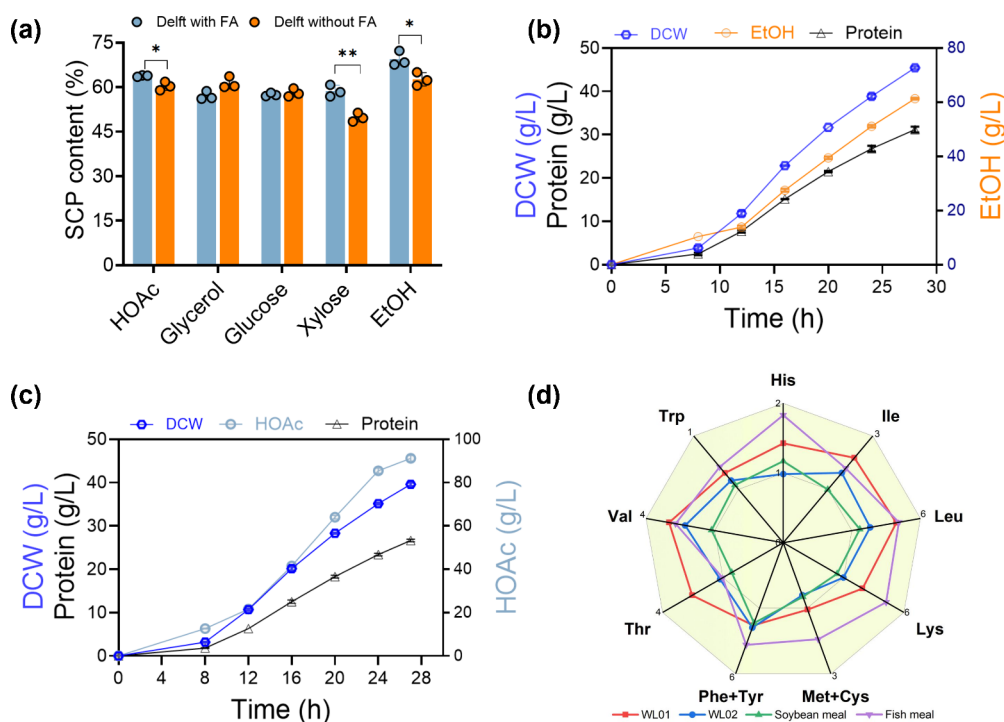


Figure 4. Using non-food carbon sources for microbial production of SCP biomass. (a) Comparison of SCP content in flask cultures using various carbon sources. All data represent the mean $\pm$ s.d. ($n = 3$ biologically independent samples) (b) DCW and cumulative EtOH consumption of CGMCC34730 over 28 h in a 5-L bioreactor. (c) DCW and cumulative HOAc consumption of CGMCC34730 over 27 h in a 5-L bioreactor. All data represent the mean $\pm$ s.d. ($n = 2$ biologically independent samples) (d) Radar chart of essential amino acid composition, with values expressed as mass percentages (% w/w), corresponding to grams of amino acid per 100 grams of dry cell weight (g/100 g DCW). Comparison of essential amino acid content of WL01 and WL02, fishmeal, and soybean meal standards. Data for fishmeal standard (5-13-0045) and soybean meal standard (5-10-0102) were obtained from China Feed Data. Statistical analysis was performed using a two-tailed Student's *t*-test (* $P < 0.05$, ** $P < 0.01$).

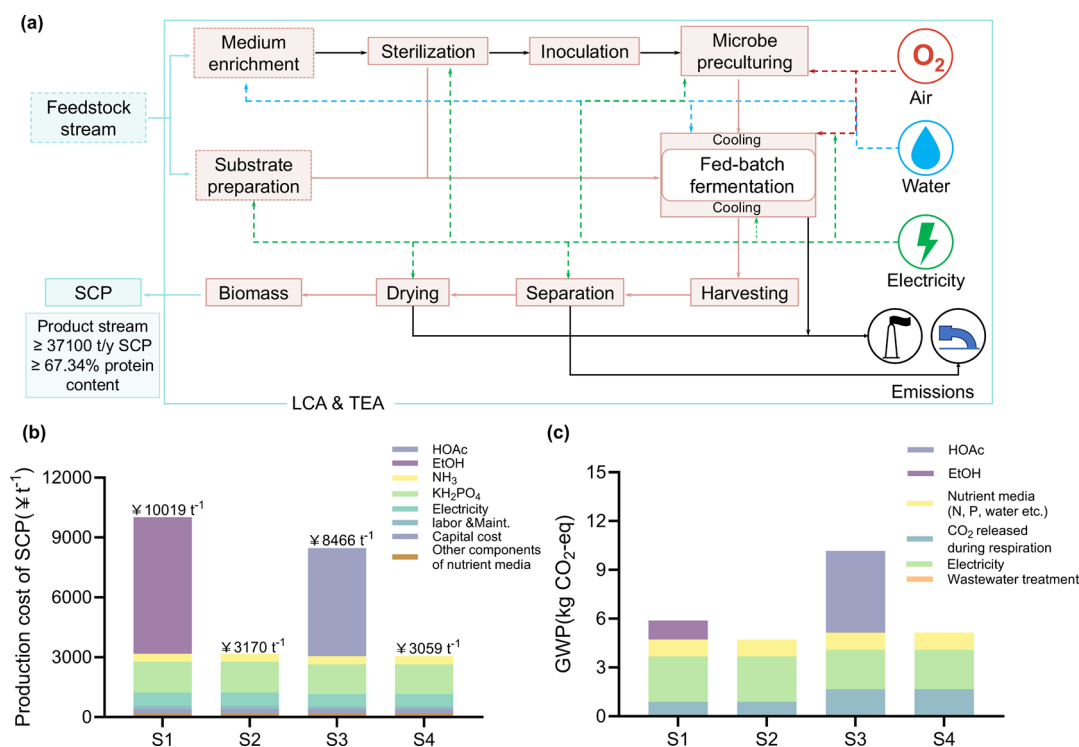


Figure 5. Techno-economic analysis (TEA) and life cycle assessment (LCA) of single-cell protein (SCP) production using EtOH and HOAc. (a) Process design and system boundaries for the baseline SCP production scenario. Dashed lines and boxes indicate process inputs; solid lines indicate outputs. (b) Comparative assessment of the leveled cost of SCP production across baseline scenarios (S1–S4). (c) Contribution analysis of the cradle-to-gate global warming potential (GWP) associated with producing 1 kg of SCP.

of 9289–11,680 CNY/t SCP.^{72,73} Significantly, the production cost of S3 (8466 CNY/t SCP) was lower than that of S1 (10019 CNY/t SCP) in commercial feedstock scenarios. This economic viability is attributed to the dual advantages of HOAc: a lower feedstock unit cost and a higher system throughput (4.84 t h^{-1}) than those of the EtOH-based process (4.63 t h^{-1}) (Tables S7 and S8). Consequently, these results demonstrate that producing SCP from HOAc offers clear economic competitiveness.

The cost breakdown (Figure 5b) reveals that KH_2PO_4 and NH_3 constitute over 60% of the total expenses in S2 and S4, suggesting that media optimization is critical for further cost reduction. Although electricity accounts for less than 23% of the total cost, cooling accounts for 46% of the energy consumption due to the substantial metabolic heat generated during fermentation. These findings underscore the importance of strategic site selection—specifically in regions with low ambient temperatures, such as northern China—to minimize cooling loads and enhance economic competitiveness. Under current market conditions, the production cost of the SCP derived from S3 is estimated at 8466 CNY/t SCP, which is lower than that of S1 (10019 CNY/t SCP). Theoretically, transitioning to wastewater-derived HOAc could reduce costs to 3059 CNY/t SCP by minimizing raw material expenditures, if pretreatment and downstream processing loads remain economically viable. Even when relying on commercial HOAc, the scenario demonstrates robust economic resilience as the projected cost creates a competitive buffer against market volatility compared with animal proteins, such as fishmeal ($>18,000 \text{ CNY/t}$

SCP, obtained from market research). Although this current cost remains higher than that of plant proteins such as high-protein soybean meal ($\geq 3100 \text{ CNY/t}$, obtained from market research), the theoretical cost achieved via wastewater-derived HOAc (3059 CNY/t SCP) or EtOH (3170 CNY/t SCP) would render this SCP economically competitive even against these inexpensive plant sources. Furthermore, the current cost significantly undercuts other microbial protein benchmarks. These established platforms include filamentous fungi valorizing wheat straw (42,224–73,080 CNY/t SCP), hydrogen-oxidizing bacteria utilizing CO_2 and electrolytic hydrogen (12,992–30,856 CNY/t SCP), microalgae cultivated on waste gas CO_2 (10,556–33,292 CNY/t SCP), and methane-oxidizing bacteria converting natural gas (24,360–40,600 CNY/t SCP).⁷⁴ Crucially, the metabolic plasticity of *C. jadinii* CGMCC34730 extends beyond C_2 substrates to encompass C_1 – C_6 carbon sources, offering avenues for further cost reduction. For instance, the co-utilization of corn cob residues, an industrial waste with a feedstock cost below 500 CNY/t, could theoretically reduce raw material expenditures,⁷⁵ thereby amplifying the economic advantage over mono-substrate-dependent systems.

In addition to economic feasibility, we conducted an LCA to evaluate the global warming potential (GWP) of producing 1 kg of SCP. The GWP for the wastewater HOAc scenario (S4) was $5.2 \text{ kg CO}_2\text{-eq}$ (Figure 5c), which was slightly higher than that of the wastewater EtOH scenario (S2) at $4.7 \text{ kg CO}_2\text{-eq}$. Contribution analysis identified electricity consumption as the primary environmental hotspot, accounting for 47% of the

Table 1. Performance Comparison of SCP Production by Different Microbial Chassis Using Non-Food Carbon Sources

microorganism	non-food carbon sources	output	reference
<i>Cupriavidus necator</i> H16	CO ₂ and H ₂	Crude protein content: 60% Protein concentration: 13.8 g L ⁻¹ Productivity: 2.76 g L ⁻¹ d ⁻¹	80
<i>Methylovibrioformis</i> <i>sanxanigenens</i>	CH ₄	Crude protein content: 34.7% Biomass: 6.94 g L ⁻¹ Productivity: 3.97 g L ⁻¹ d ⁻¹	81
<i>Hydrogenophaga pseudoflava</i> Z-1107	CO and H ₂	Crude protein content: 67.2% Biomass: 0.57 g L ⁻¹	82
<i>Clostridium autoethanogenum</i>	CO/CO ₂	Crude protein content: 70~80% Biomass: 20~25 g L ⁻¹	17
<i>Microalgae Chlorella vulgaris</i>	CO ₂	Crude protein content: 61.6% Biomass: 0.62 g L ⁻¹	83
<i>Yarrowia lipolytica</i>	Oat bran hydrolysate	Crude protein content: 45% Biomass: 9.35 g L ⁻¹ Productivity: 1.87 g d ⁻¹	84
<i>Yarrowia lipolytica</i>	waste cooking oils	Crude protein content: 12.6% Protein concentration: 7.23 g L ⁻¹ Biomass: 57.37 g L ⁻¹	85
<i>Pichia pastoris</i>	methanol and glycerol	Crude protein content: 52% Biomass: 63.37 g L ⁻¹ Productivity: 21.12 g L ⁻¹ d ⁻¹	10
<i>Rhodotorula glutinis</i>	vinegar residue	Crude protein content: 37.17% Biomass: 32.14 g L ⁻¹ Protein concentration: 8.5 g L ⁻¹	72
<i>Kluyveromyces marxianus</i>	corn straw	Crude protein content: 57.3% Biomass: 5.3 g L ⁻¹	6
<i>Candida utilis</i>	dihydroxyacetone	Crude protein content: 60.1% Biomass: 34.8 g L ⁻¹ Productivity: 1.3 g L ⁻¹ d ⁻¹	7
	HOAc	Crude protein content: 45% Protein concentration: 1.54 g L ⁻¹ EAAI: 76.4% Yield: 0.38 g DCW g ⁻¹	
	EtOH	Crude protein content: 0.54 g protein (g _{biomass}) ⁻¹ (≈27 g L ⁻¹) Biomass: 100 g _{biomass} (kg _{broth}) ⁻¹ Biomass Yield: 0.65 g _{biomass} (g _{ethanol}) ⁻¹	28
<i>C. jadinii</i> CGMCC34730	EtOH	Crude protein content: 68.6% Biomass: 45.45 g L ⁻¹ Biomass productivity: 38.64 g L ⁻¹ d ⁻¹ EAAI: 97%	this work
	HOAc	Crude protein content: 67.34% Biomass: 39.60 g L ⁻¹ Biomass productivity: 35.04 g L ⁻¹ d ⁻¹ EAAI: 75%	this work

total impact, followed by metabolic respiration (38%). While this study assumed the use of wind power, further reductions in emissions could be achieved by transitioning to nuclear energy or enhancing renewable efficiency.⁷⁶ Although the carbon footprint of S4 is higher than that of soy protein concentrate (0.8 kg CO₂-eq) and fishmeal (1.5 kg CO₂-eq),⁷⁷ it remains lower than that of alternative proteins such as FeedKind (8.0 kg CO₂-eq) and mycoprotein (15–38.4 kg CO₂-eq).^{78,79} However, comparing LCA results across different studies is complex, as each author adopts different methodological approaches, system boundaries, and assumptions. Since ex-ante LCA for early-stage technologies heavily relies on scaling estimates and projections, these results should be interpreted as indicators of potential future impacts, highlighting the need for continuous methodological refinement and optimization as the process matures. Overall, the use of wastewater-derived substrates offers dual benefits: substantially lower costs and carbon footprints than commercial substrates, and environmental performance comparable to that of

established protein sources, thereby supporting circular economy objectives.

4. CONCLUSIONS

The efficient utilization of non-food substrates by *C. jadinii* CGMCC34730 presents a promising alternative to traditional sugar-based biomanufacturing. In this study, compared with other carbon sources, C2 substrates demonstrated superior performance in supporting SCP production. This metabolic capability is particularly significant as it facilitates the valorization of electrochemically derived HOAc from CO₂. Furthermore, the broad substrate range of *C. jadinii* CGMCC34730 indicates the potential for wide applicability in upgrading diverse industrial streams, including lignocellulosic hydrolysates and corn steep liquor. TEA confirms the viability and sustainability of *C. jadinii* CGMCC34730-based SCP production platform; projected costs are highly competitive against current market prices, supporting both scalability and long-term commercial sustainability. Thus,

this platform provides a viable, land-independent strategy for converting non-food resources into value-added SCP.

■ ASSOCIATED CONTENT

Data Availability Statement

Whole-genome sequencing (WGS) data for 20 samples of *C. jadinii* generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1244612. The WGS data of the strain CGMCC34730, along with the RNA-seq data generated in this study, have been deposited in the Genome Sequence Archive⁸⁶ at the National Genomics Data Center⁸⁷ under accession numbers CRA031663 and CRA031991, respectively, and are publicly accessible at <https://ngdc.cnbc.ac.cn/gsa>.

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssuschemeng.6c02957>.

Strains and plasmids used in this study (Table S1); primers used in this study (Table S2); sequences of the genes involved in this study (Table S3); instrument parameters in TEA analysis (Table S4); instrument parameters in TEA analysis (Table S5); system capital cost in TEA analysis (Table S6); total cost calculation for SCP production using EtOH as the sole carbon source in TEA analysis (Table S7); total cost calculation for SCP production using HOAc as the sole carbon source in TEA analysis (Table S8); growth of CGMCC34730 using EtOH or HOAc as carbon sources (Table S9); nutritional evaluation of essential amino acids: EAAI (Table S10); cellular composition of EtOH/HOAc-driven SCP (Table S11); ¹³C_{TOC} content analysis of the biomass using ¹³C-labelled HOAc as the sole carbon source (Table S12); list of abbreviations (Table S13); process of gene genetic manipulation in *C. jadinii* CGMCC34730 (Figure S1); example of labeling for glucose-6-phosphate (Figure S2); NADH levels in *C. jadinii* CGMCC34730 cultivated on different carbon sources (Figure S3); carbon source characterization of isolated *C. jadinii* CGMCC34730 (Figure S4); ¹³C-isotopic analysis of the metabolites in CGMCC34730 during FA fermentation (Figure S5); transcriptomic analysis of the CGMCC34730 cultured in FA-containing medium (Figure S6); endogenous FA assimilation pathways in CGMCC34730 (Figure S7); growth profiles of the engineered strains related to FA utilization (Figure S8); comparison of SCP production in flask cultures (40 mL) using various carbon sources (Figure S9); amino acid profile of SCP produced by strain CGMCC34730 cultured in EtOH (WL01) or HOAc (WL02) as the sole carbon source (Figure S10); growth curves of CGMCC34730 utilizing commercial HOAc and CO₂-derived HOAc as the sole carbon source (Figure S11); Supplementary Method 1: gene editing and identification; Supplementary Method 2: whole-genome sequencing and data analysis; and Supplementary Method 3: transcriptome Profiling (DOCX)

■ AUTHOR INFORMATION

Corresponding Authors

Chuan Xia – School of Materials and Energy, University of Electronic Science and Technology of China, Chengdu 611731,

China; orcid.org/0000-0003-4526-159X;
Email: chuan.xia@uestc.edu.cn. Tel: +86-15011369369;
Fax: +86-15011369369.

Yajie Wang – School of Engineering, Westlake Center of Synthetic Biology and Integrated Bioengineering, Westlake University, Hangzhou 310030, China; Center for Future Food, Muyuan Laboratory, Zhengzhou 450007, China; School of Life Science, Westlake University, Hangzhou 310030, China; orcid.org/0009-0002-1903-6786;
Email: wangyajie@westlake.edu.cn.
Tel: +86-19857111775

Authors

Wei Zhong – School of Engineering, Westlake Center of Synthetic Biology and Integrated Bioengineering, Westlake University, Hangzhou 310030, China

Chong Xie – Center for Future Food, Muyuan Laboratory, Zhengzhou 450007, China; Henan Academy of Sciences, Zhengzhou 450046, China

Kuan Chen – School of Life Science, Westlake University, Hangzhou 310030, China

Nana Liu – School of Engineering, Westlake Center of Synthetic Biology and Integrated Bioengineering, Westlake University, Hangzhou 310030, China

Chengchao Zhu – Center for Future Food, Muyuan Laboratory, Zhengzhou 450007, China

Junze Liu – Center for Future Food, Muyuan Laboratory, Zhengzhou 450007, China

Huiqi Sun – School of Engineering, Westlake Center of Synthetic Biology and Integrated Bioengineering, Westlake University, Hangzhou 310030, China

Jingliang Xu – School of Chemical Engineering, Zhengzhou University, Zhengzhou, Henan 450001, China; orcid.org/0000-0003-0703-6631

Lei Wang – Key Laboratory of Coastal Environment and Resources of Zhejiang Province, School of Engineering, Westlake University, Hangzhou, Zhejiang 310024, China; orcid.org/0000-0003-4128-687X

Complete contact information is available at:
<https://pubs.acs.org/doi/10.1021/acssuschemeng.6c02957>

Author Contributions

*W.Z. and C.X. contributed equally to this work. Y.W., W.Z., C.X.¹ (Chong Xie), and C.X.² (Chuan Xia) conceived and designed the study. Y.W. supervised the project. W.Z., K.C., and N.L. performed the isolation and carbon-source characterization experiments and the mechanism experiments on FA as an auxiliary substrate for growth promotion. C.X.¹, C.Z., W.Z., J.L., and H.S. were involved in strain fermentation and SCP detection. C.X.¹ and H.S. performed the techno-economic analysis, life cycle assessment, and cellular composition analysis. C.X.² (Chuan Xia) provided the electrocatalysis system used to produce EtOH and HOAc from CO₂. W.Z., C.X.¹, C.X.², and Y.W. wrote and revised the manuscript with input of all authors. C.X.², L.W., and J.X. contributed to the review, editing, and final approval of the manuscript. They provided valuable insights and suggestions to improve the quality of the research paper. All authors read and revised the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (32401209), National Key R&D Program of China (2022YFA0912800), Westlake University-Muyuan Joint Research Institute (Program ID: WU2025MY006), and the Muyuan Laboratory Research and Innovation Project (11146022401). C.X.² acknowledges the Scientific Research Innovation Capability Support Project for Young Faculty (SRICSPYF-ZY2025052), the Natural Science Foundation of Sichuan Province (2025NSFJQ0017), and the University of Electronic Science and Technology of China (ZYGX2025TS001). We thank Wenwen Zhang and Xiaoyan Xu (Mass Spectrometry & Metabolomics Core Facility of Westlake University) for the MS analysis.

■ REFERENCES

- (1) United Nations Revision of World Population Prospects (2024) <https://population.un.org/wpp/> (accessed 2026-07-05).
- (2) Food and Agriculture Organization of the United Nations. *The State of Food Security and Nutrition in the World 2025* <https://www.fao.org/news/story/en/item/35571/icode/> (accessed 2026-07-05).
- (3) Protein Sources, 2025. *Global protein supply—an overview*. <https://foodtechnologies.messefrankfurt.com/global/en/news-insights/articles/global-protein-supply.html> (accessed 2026-07-05).
- (4) Henschion, M.; Hayes, M.; Mullen, A. M.; Fenelon, M.; Tiwari, B. Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. *Foods* **2017**, *6* (7), 53.
- (5) Ariëns, R. M. C.; Bastiaan-Net, S.; van de Berg-Somhorst, D. B. P. M.; El Bachrioui, K.; Boudewijn, A.; van den Dool, R. T. M.; de Jong, G. A. H.; Wichers, H. J.; Mes, J. J. Comparing nutritional and digestibility aspects of sustainable proteins using the INFOGEST digestion protocol. *J. Funct. Foods* **2021**, *87*, 104748.
- (6) Ji, Z.; Du, G.; Li, J.; Zhang, F.; Meng, Z.; Fu, G.; Lin, Y.; Gong, D.; Lee, S. Y.; Zhang, D. Single-Cell Protein Production from Corn Straw by *Kluyveromyces marxianus* Evolved through Integrated Adaptive Evolution and Physical Mutagenesis. *ACS Sustainable Chem. Eng.* **2025**, *13* (50), 21510–21523.
- (7) Zhang, Y.; Cao, X.; Wang, W.; Li, C. Synthesis of single cell protein from C1-derived dihydroxyacetone by *Candida utilis*. *Syst. Microbiol. Biomanuf.* **2025**, *5* (2), 667–678.
- (8) Jach, M. E.; Serefko, A.; Ziaja, M.; Kieliszek, M. Yeast Protein as an Easily Accessible Food Source. *Metabolites* **2022**, *12*, 63.
- (9) Ma, J.; Sun, Y.; Meng, D.; Zhou, Z.; Zhang, Y.; Yang, R. Yeast proteins: The novel and sustainable alternative protein in food applications. *Trends Food Sci. Tech.* **2023**, *135*, 190–201.
- (10) Meng, J.; Liu, S.; Gao, L.; Hong, K.; Liu, S.; Wu, X. Economical production of *Pichia pastoris* single cell protein from methanol at industrial pilot scale. *Microb. Cell Fact.* **2023**, *22* (1), 198.
- (11) Gao, L.; Meng, J.; Dai, W.; Zhang, Z.; Dong, H.; Yuan, Q.; Zhang, W.; Liu, S.; Wu, X. Deciphering cell wall sensors enabling the construction of robust *P. pastoris* for single-cell protein production. *Biotechnol. Biofuels Bioprod.* **2023**, *16* (1), 178.
- (12) Finnigan, T. J. A.; Theobald, H. E.; Bajka, B. Mycoprotein: A Healthy and Sustainable Source of Alternative Protein-Based Foods. *Annu. Rev. Food Sci. Technol.* **2025**, *16*, 105–125.
- (13) Gada, P.; Moreira, C.; Silva, M.; Nunes, R.; Madureira, L.; Rocha, C. M. R.; Pereira, R. N.; Vicente, A. A.; Teixeira, J. A. Algal proteins: Production strategies and nutritional and functional properties. *Bioresour. Technol.* **2021**, *332*, 125125.
- (14) Gordalina, M.; Pinheiro, H. M.; Mateus, M.; Fonseca, M. M. R.; da; Cesário, M. T. Macroalgae as Protein Sources—A Review on Protein Bioactivity, Extraction, Purification and Characterization. *Appl. Sci.* **2021**, *11*, 7969.
- (15) Ukaegbu-Obi, K. M. Single Cell Protein: A Resort to Global Protein Challenge and Waste Management. *J. Microbiol. Microb. Technol.* **2016**, *1* (1), 5.
- (16) Zhu, Z.; Wu, Y.; Hu, W.; Zheng, X.; Chen, Y. Valorization of food waste fermentation liquid into single cell protein by photosynthetic bacteria via stimulating carbon metabolic pathway and environmental behaviour. *Bioresour. Technol.* **2022**, *361*, 127704.
- (17) Ma, S.; Liang, X.; Chen, P.; Wang, J.; Gu, X.; Qin, Y.; Blecker, C.; Xue, M. A new single-cell protein from *Clostridium autoethanogenum* as a functional protein for largemouth bass (*Micropterus salmoides*). *Anim. Nutr.* **2022**, *10*, 99–110.
- (18) Pan, Z.; Hu, X.; Guo, Y.; Yu, J.; Zhang, H.; Guo, K. Simultaneous production, concentration, and upgradation of acetate into single-cell protein in a hybrid microbial electrochemical system. *Chem. Eng. J.* **2025**, *503*, 158446.
- (19) Thomas, A. B.; Shetane, T. D.; Singha, R. G.; Nanda, R. K.; Poddar, S. S.; Shirsat, A. Employing Central Composite Design for Evaluation of Biomass Production by *Fusarium venenatum*: In Vivo Antioxidant and Antihyperlipidemic Properties. *Appl. Biochem. Biotech.* **2017**, *183* (1), 91–109.
- (20) Jach, M. E.; Baj, T.; Juda, M.; Swider, R.; Mickowska, B.; Malm, A. Statistical evaluation of growth parameters in biofuel waste as a culture medium for improved production of single cell protein and amino acids by *Yarrowia lipolytica*. *AMB Exp.* **2020**, *10* (1), 35.
- (21) Watteuw, C. M.; Armiger, W. B.; Ristroph, D. L.; Humphrey, A. E. Production of single cell protein from ethanol by fed-batch process. *Biotechnol. Bioeng.* **1979**, *21* (7), 1221–1237.
- (22) Pessarrodona, A.; Franco-Santos, R. M.; Wright, L. S.; Vanderklift, M. A.; Howard, J.; Pidgeon, E.; Wernberg, T.; Filbee-Dexter, K. Carbon sequestration and climate change mitigation using macroalgae: a state of knowledge review. *Biol. Rev.* **2023**, *98* (6), 1945–1971.
- (23) Chilakamarri, C. R.; Khilji, I. A.; Sirohi, R.; Pandey, A.; Baskar, G.; Satyavolu, J. Maximizing the value of biodiesel industry waste: Exploring recover, recycle, and reuse for sustainable environment. *Environ. Technol. Innov.* **2023**, *32*, 103447.
- (24) Li, P.; He, C.; Li, G.; Ding, P.; Lan, M.; Gao, Z.; Jiao, Y. Biological pretreatment of corn straw for enhancing degradation efficiency and biogas production. *Bioengineered* **2020**, *11* (1), 251–260.
- (25) Al-Breiki, M.; Bicer, Y. In *Sustainable Energy Carriers for Energy Storage and Transport: Exploring Advanced Solutions for a Green Future*, Al-Breiki, M.; Bicer, Y., Eds.; Springer Nature Switzerland: Cham, **2025**; pp 83–104.
- (26) Mengqin, W.; Jiayao, W.; Youqiang, X.; Yu, W. Progress in cascade conversion of CO₂ to single cell protein through chemical and biological catalysis. *Chem. Ind. Eng. Prog.* **2025**, *44* (5), 2429–2440.
- (27) Herman, N. A.; Kim, S. J.; Li, J. S.; Cai, W.; Koshino, H.; Zhang, W. The industrial anaerobe *Clostridium acetobutylicum* uses polyketides to regulate cellular differentiation. *Nat. Commun.* **2017**, *8* (1), 1514.
- (28) Vieira-Lara, M. A.; Warmerdam, M.; de Hulster, E. A. F.; van den Broek, M.; Daran, J.-M.; Pronk, J. T. Quantitative physiology and biomass composition of *Cyberlindnera jadinii* in ethanol-grown cultures. *Biotechnol. Biofuels Bioprod.* **2024**, *17* (1), 142.
- (29) Gu, L.; Zhang, R.; Fan, X.; Wang, Y.; Ma, K.; Jiang, J.; Li, G.; Wang, H.; Fan, F.; Zhang, X. Development of CRISPR/Cas9-Based Genome Editing Tools for Polyploid Yeast *Cyberlindnera jadinii* and Its Application in Engineering Heterologous Steroid-Producing Strains. *ACS Synth. Biol.* **2023**, *12* (10), 2947–2960.
- (30) van Urk H.; Postma, E.; Scheffers, W. A.; van Dijken J. P. Glucose Transport in Crabtree-positive and Crabtree-negative Yeasts. *Microbiology* **1989**, *135* (9), 2399–2406.
- (31) Tamakawa, H.; Mita, T.; Yokoyama, A.; Ikushima, S.; Yoshida, S. Metabolic engineering of *Candida utilis* for isopropanol production. *Appl. Microbiol. Biotechnol.* **2013**, *97* (14), 6231–6239.
- (32) Tamakawa, H.; Ikushima, S.; Yoshida, S. Efficient production of l-lactic acid from xylose by a recombinant *Candida utilis* strain. *J. Biosci. Bioeng.* **2012**, *113* (1), 73–75.

- (33) Kot, A. M.; Błażej, S.; Nosek, K.; Synowiec, A.; Chlebowska-Śmigiel, A.; Pobiega, K. Magnesium Binding by *Cyberlindnera jadinii* Yeast in Media from Potato Wastewater and Glycerol. *Microorganisms* **2023**, *11*, 1923.
- (34) Lim, H. G.; Lee, J. H.; Noh, M. H.; Jung, G. Y. Rediscovering Acetate Metabolism: Its Potential Sources and Utilization for Biobased Transformation into Value-Added Chemicals. *J. Agric. Food Chem.* **2018**, *66* (16), 3998–4006.
- (35) Schmid, R.; Blaxter, M. L. annot8r: GO, EC and KEGG annotation of EST datasets. *BMC Bioinf.* **2008**, *9* (1), 180.
- (36) Wang, D.; Li, D.; Zhang, G.; Wang, C.; Wei, G. Disruption of *por1* gene in *Candida utilis* improves co-production of S-adenosylmethionine and glutathione. *J. Biotechnol.* **2019**, *290*, 16–23.
- (37) Zhang, J.; Wei, G.; Dong, H.; Zhu, T.; Li, Y. A new knocking-out system in *Candida utilis* and its application on disrupting the *gsh1* gene. *Microbiol. China* **2011**, *38* (6), 795–802.
- (38) Gietz, R. D.; Woods, R. A. Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. *Method. Enzymol.* **2002**, *350*, 87–96.
- (39) Zhong, W.; Liu, N.; Chen, B.; Sun, H.; Fei, X.; Lian, J.; Guo, J.; Wang, B.; Wang, Y. Engineering energy-efficient *Saccharomyces cerevisiae* for methanol and CO₂ assimilation. *Nat. Commun.* **2026**, *17* (1), 1806.
- (40) Wang, Y.; Parsons, L. R.; Su, X. AccuCor2: isotope natural abundance correction for dual-isotope tracer experiments. *Lab. Invest.* **2021**, *101* (10), 1403–1410.
- (41) Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A. A.; Dvorkin, M.; Kulikov, A. S.; Lesin, V. M.; Nikolenko, S. I.; Pham, S.; Pribelski, A. D.; Pyshkin, A. V.; Sirotkin, A. V.; Vyahhi, N.; Tesler, G.; Alekseyev, M. A.; Pevzner, P. A. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **2012**, *19* (5), 455–477.
- (42) Boetzer, M.; Pirovano, W. Toward almost closed genomes with GapFiller. *Genome Biol.* **2012**, *13* (6), R56.
- (43) Tatusov, R. L.; Galperin, M. Y.; Natale, D. A.; Koonin, E. V. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res.* **2000**, *28* (1), 33–36.
- (44) Urban, M.; Pant, R.; Raghunath, A.; Irvine, A. G.; Pedro, H.; Hammond-Kosack, K. E. The Pathogen-Host Interactions database (PHI-base): additions and future developments. *Nucleic Acids Res.* **2015**, *43* (Issue D1), D645–D655.
- (45) Lombard, V.; Golaconda Ramulu, H.; Drula, E.; Coutinho, P. M.; Henrissat, B. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res.* **2013**, *42* (D1), D490–D495.
- (46) Ranallo-Benavidez, T. R.; Jaron, K. S.; Schatz, M. C. GenomeScope 20 and Smudgeplot for reference-free profiling of polyploid genomes. *Nat. Commun.* **2020**, *11* (1), 1432.
- (47) Pertea, M.; Pertea, G. M.; Antonescu, C. M.; Chang, T.-C.; Mendell, J. T.; Salzberg, S. L. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat. Biotechnol.* **2015**, *33* (3), 290–295.
- (48) Shen, S.; Park, J. W.; Lu, Z.-x.; Lin, L.; Henry, M. D.; Wu, Y. N.; Zhou, Q.; Xing, Y. rMATS: Robust and flexible detection of differential alternative splicing from replicate RNA-Seq data. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (S1), E5593–E5601.
- (49) Wu, J.; Ohnuki, S.; Teyssonnière, É.; Yasukawa, T.; Masuo, N.; Schacherer, J.; Ohya, Y. Genetic and phenotypic insights into *Cyberlindnera jadinii* as a promising yeast for industrial biotechnology. *G3-GENES Genom. Genet.* **2025**, *12*, jkaf145.
- (50) Zhang, C.; Fu, R.; Kang, L.; Ma, Y.; Fan, D.; Fei, Q. An upcycling bioprocess for sustainable aviation fuel production from food waste-derived greenhouse gases: Life cycle assessment and techno-economic analysis. *Chem. Eng. J.* **2024**, *486*, 50242.
- (51) Guinée, J. Handbook on life cycle assessment: Operational guide to the ISO standards. *Int. J. Life Cycle Ass.* **2001**, *6* (5), 255.
- (52) Zhu, Z.; Geng, P.; Li, M.; Yin, H.; Li, C.; Cao, X.; Tian, J. A new strategy of carbon-energy coupling transfer enhancement by formate on phototrophic green microalga *Chlamydomonas reinhardtii*. *Chem. Eng. J.* **2024**, *488*, 151182.
- (53) Sáez-Plaza, P.; Michałowski, T.; Navas, M. J.; Asuero, A. G.; Wybraniec, S. An Overview of the Kjeldahl Method of Nitrogen Determination. Part I Early History, Chemistry of the Procedure, and Titrimetric Finish. *Crit. Rev. Anal. Chem.* **2013**, *43* (4), 178–223.
- (54) Ali, A. A. M.; Noor, H. S. M.; Chong, P. K.; Babji, A. S.; Lim, S. J. Comparison of amino acids profile and antioxidant activities between edible bird nest and chicken egg. *Malays. Appl. Biol.* **2019**, *48* (2), 63–69.
- (55) Sun, P.; Zhang, H.; Sun, Y.; Liu, J. The recent development of fluorescent probes for the detection of NADH and NADPH in living cells and in vivo. *Spectrochim. Acta A* **2021**, *245*, 118919.
- (56) Hayakawa, K.; Matsuda, F.; Shimizu, H. ¹³C-metabolic flux analysis of ethanol-assimilating *Saccharomyces cerevisiae* for S-adenosyl-methionine production. *Microb. Cell Fact.* **2018**, *17* (1), 82.
- (57) Gao, J.; Yu, W.; Li, Y.; Jin, M.; Yao, L.; Zhou, Y. J. Engineering co-utilization of glucose and xylose for chemical overproduction from lignocellulose. *Nat. Chem. Biol.* **2023**, *19* (12), 1524–1531.
- (58) Tamakawa, H.; Ikushima, S.; Yoshida, S. Ethanol Production from Xylose by a Recombinant *Candida utilis* Strain Expressing Protein-Engineered Xylose Reductase and Xylitol Dehydrogenase. *Biosci., Biotechnol., Biochem.* **2011**, *75* (10), 1994–2000.
- (59) Bzducha-Wróbel, A.; Pobiega, K.; Błażej, S.; Kieliszek, M. The scale-up cultivation of *Candida utilis* in waste potato juice water with glycerol affects biomass and β(1,3)/(1,6)-glucan characteristic and yield. *Appl. Microbiol. Biotechnol.* **2018**, *102* (21), 9131–9145.
- (60) Bruinenberg, P. M.; Jonker, R.; Dijken, J. P., van; Scheffers, W. A. Utilization of formate as an additional energy source by glucose-limited chemostat cultures of *Candida utilis* CBS 621 and *Saccharomyces cerevisiae* CBS 8066. *Arch. Microbiol.* **1985**, *142* (3), 302–306.
- (61) Bysani, V. R.; Alam, A. M.; Bar-Even, A.; Machens, F. Engineering and evolution of the complete Reductive Glycine Pathway in *Saccharomyces cerevisiae* for formate and CO₂ assimilation. *Metab. Eng.* **2023**, *81*, 167.
- (62) Ploessl, D.; Zhao, Y.; Cao, M.; Ghosh, S.; Lopez, C.; Sayadi, M.; Chudalayandi, S.; Severin, A.; Huang, L.; Gustafson, M.; Shao, Z. A repackaged CRISPR platform increases homology-directed repair for yeast engineering. *Nat. Chem. Biol.* **2022**, *18* (1), 38–46.
- (63) Yang, Z.; Blenner, M. Genome editing systems across yeast species. *Curr. Opin. Biotechnol.* **2020**, *66*, 255–266.
- (64) Mitic, B. M.; Troyer, C.; Lutz, L.; Baumschabl, M.; Hann, S.; Mattanovich, D. The oxygen-tolerant reductive glycine pathway assimilates methanol, formate and CO₂ in the yeast *Komagataella phaffii*. *Nat. Commun.* **2023**, *14* (1), 7754.
- (65) Claassens, N. J.; Bordanaba-Florit, G.; Cotton, C. A. R.; De Maria, A.; Finger-Bou, M.; Friedenheim, L.; Giner-Laguada, N.; Munar-Palmer, M.; Newell, W.; Scarinci, G.; Verbunt, J.; Vries, S. T., de; Yilmaz, S.; Bar-Even, A. Replacing the Calvin cycle with the reductive glycine pathway in *Cupriavidus necator*. *Metab. Eng.* **2020**, *62*, 30–41.
- (66) Sánchez-Andrea, I.; Guedes, I. A.; Hornung, B.; Boeren, S.; Lawson, C. E.; Sousa, D. Z.; Bar-Even, A.; Claassens, N. J.; Stams, A. J. M. The reductive glycine pathway allows autotrophic growth of *Desulfovibrio desulfuricans*. *Nat. Commun.* **2020**, *11* (1), 5090.
- (67) de Mello, A. F. M.; de Souza Vandenbergh, L. P.; Herrmann, L. W.; Letti, L. A. J.; Burgos, W. J. M.; Scapini, T.; Manzoki, M. C.; de Oliveira, P. Z.; Socol, C. R. Strategies and engineering aspects on the scale-up of bioreactors for different bioprocesses. *Syst. Microbiol. Biom.* **2024**, *4* (2), 365–385.
- (68) Mahmoodi, M.; Nassireslami, E. Control algorithms and strategies of feeding for fed-batch fermentation of *Escherichia coli*: a review of 40 years of experience. *Prep. Biochem. Biotech.* **2022**, *52* (7), 823–834.
- (69) Leger, D.; Matassa, S.; Noor, E.; Shepon, A.; Milo, R.; Bar-Even, A. Photovoltaic-driven microbial protein production can use land and sunlight more efficiently than conventional crops. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118* (26), e2015025118.

- (70) Zheng, T.; Zhang, M.; Wu, L.; Guo, S.; Liu, X.; Zhao, J.; Xue, W.; Li, J.; Liu, C.; Li, X.; Jiang, Q.; Bao, J.; Zeng, J.; Yu, T.; Xia, C. Upcycling CO₂ into energy-rich long-chain compounds via electrochemical and metabolic engineering. *Nat. Catal.* **2022**, *5* (5), 388–396.
- (71) Market Growth Reports; Single Cell Protein Products Market Size, Share, Growth, and Industry Analysis, By Type (Yeast, Algae, Bacteria, Others), By Application (Animal Feed, Human Food), Regional Insights and Forecast to 2033, **2023**; <https://www.marketgrowthreports.com/market-reports/single-cell-protein-products-market-107608>.
- (72) Li, Y.; Wang, C.; Huang, R.; Zhang, S.; Yin, J.; Zhang, J.; Wu, J. Production of single-cell protein from vinegar residue by *Rhodotorula glutinis* and techno-economic analysis. *Bioresour. Technol.* **2025**, *422*, 132252.
- (73) El Abbadi, S. H.; Sherwin, E. D.; Brandt, A. R.; Luby, S. P.; Criddle, C. S. Displacing fishmeal with protein derived from stranded methane. *Nat. Sustain.* **2022**, *5* (1), 47–56.
- (74) Ye, L.; Bogicevic, B.; Bolten, C. J.; Wittmann, C. Single-cell protein: overcoming technological and biological challenges towards improved industrialization. *Curr. Opin. Biotechnol.* **2024**, *88*, 103171.
- (75) Yan, X.; Bao, W.; Wu, Y.; Zhang, C.; Mao, Z.; Yuan, Q.; Hu, Z.; He, P.; Peng, Q.; Hu, M.; Geng, B.; Ma, H.; Chen, S.; Fei, Q.; He, Q.; Yang, S. Paradigm of engineering recalcitrant non-model microorganism with dominant metabolic pathway as a biorefinery chassis. *Nat. Commun.* **2024**, *15* (1), 10441.
- (76) Khoshnevisan, B.; Tabatabaei, M.; Tsapekos, P.; Rafiee, S.; Aghbashi, M.; Lindene, S.; Angelidaki, I. Environmental life cycle assessment of different biorefinery platforms valorizing municipal solid waste to bioenergy, microbial protein, lactic and succinic acid. *Renew. Sustain. Energy Rev.* **2020**, *117*, 109493.
- (77) McKuin, B.; Kapuscinski, A. R.; Sarker, P. K.; Cheek, N.; Lim, J.; Sabarsky, M. Comparative life cycle assessment of marine microalgae, *Nannochloropsis* sp. and fishmeal for sustainable protein ingredients in aquaculture feeds. *Elementa-Sci. Anthropol.* **2023**, *11* (1), 00083.
- (78) Sillman, J.; Uusitalo, V.; Ruuskanen, V.; Ojala, L.; Kahiluoto, H.; Soukka, R.; Ahola, J. A life cycle environmental sustainability analysis of microbial protein production via power-to-food approaches. *Int. J. Life Cycle Assess.* **2020**, *25* (11), 2190–2203.
- (79) Peteghem, L.; Van; Sakarika, M.; Matassa, S.; Pikaar, I.; Ganigué, R.; Rabaey, K. Towards new carbon-neutral food systems: Combining carbon capture and utilization with microbial protein production. *Bioresour. Technol.* **2022**, *349*, 126853.
- (80) Ma, C.; Cheng, X.; Wang, Y.; Chen, K.; Ma, Y.; Lin, Y.; Guo, K.; Zhu, Z. High-efficiency CO₂ fixation and single-cell protein production in *Cupriavidus necator* via ATP-driven metabolic rewiring. *Green Chem.* **2026**, *28* (10), 4550–4563.
- (81) Gao, Z.; Liu, Y.; Jiao, S.; Fu, R.; Chen, Y.; Zhang, C.; Shen, X.; Yang, G.; Yang, S.; Wang, X.; Shi, L.; Fei, Q. Biological valorization of methane and nitrogen gas-derived ammonia via methanotrophic bacteria for gut-beneficial nutrients. *Nat. Commun.* **2026**, *17* (1), 3803.
- (82) Yin, B.; Jiang, Y.; Yu, M.; Yan, Z.; Fu, S.-f.; Dou, Y. Integrating Photocatalysis and Biosynthesis for Upgrading CO₂ into Single-Cell Protein. *ACS Catal.* **2026**, *16*, 4387.
- (83) Ahmad, M. T.; Shariff, M.; Md. Yusoff, F.; Goh, Y. M.; Banerjee, S. Applications of microalga *Chlorella vulgaris* in aquaculture. *Rev. Aquacult.* **2020**, *12* (1), 328–346.
- (84) Drzymala, K.; Mironczuk, A. M.; Pietrzak, W.; Dobrowolski, A. Rye and Oat Agricultural Wastes as Substrate Candidates for Biomass Production of the Non-Conventional Yeast *Yarrowia lipolytica*. *Sustainability* **2020**, *12*, 7704.
- (85) Spalvins, K.; Geiba, Z.; Kusnere, Z.; Blumberga, D. Waste Cooking Oil as Substrate for Single Cell Protein Production by Yeast *Yarrowia lipolytica*. *Environ. Clim. Technol.* **2020**, *24* (3), 457–469.
- (86) Zhang, S.; Chen, X.; Jin, E.; Wang, A.; Chen, T.; Zhang, X.; Zhu, J.; Dong, L.; Sun, Y.; Yu, C.; Zhou, Y.; Fan, Z.; Chen, H.; Zhai, S.; Sun, Y.; Chen, Q.; Xiao, J.; Song, S.; Zhang, Z.; Bao, Y.; Wang, Y.; Zhao, W. The GSA Family in 2025: A Broadened Sharing Platform for Multi-omics and Multimodal Data. *Genom. Proteom. Bioinf.* **2025**, *23* (4), qzaf072.
- (87) CNCB-NGDC Members and Partners; Database Resources of the National Genomics Data Center, China National Center for Bioinformation in 2025. *Nucleic Acids Res.* **2025**, *53* (D1), D30–D44; <https://doi.org/10.1093/nar/gkae978>.