Analysis of one-carbon compound microbial assimilation pathways and research progress in synthetic biology modification

Baoxin Zhang[1,2,3], Hailei Zhang[1,2,3], Li Zhu[2], Xiang Weng[2] & Hao Sun[1,2]

[1] Key Laboratory of Bio-Efficient Utilization of Carbon Compounds in Xinjiang Uygur Autonomous Region and the Xinjiang Synthetic Biology Industry Innovation Institute of Xinjiang Uygur Autonomous Region, Yili, China

[2] Yili Chuan Ning Biotechnology Co., Ltd, Yili, 835007, China

[3] Yili Weining Biology Co., Ltd, Yili, 835007, China

Correspondence: Baoxin Zhang, Key Laboratory of Bio-Efficient Utilization of Carbon Compounds in Xinjiang Uygur Autonomous Region and the Xinjiang Synthetic Biology Industry Innovation Institute of Xinjiang Uygur Autonomous Region, Yili, China, and Hao Sun, Yili Chuan Ning Biotechnology Co., Ltd, Yili, 835007, China. E-mail: zhangbaoxin@phabuilder.com and haosun.ucl@163.com

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Abstract

One-carbon (C1) compounds, including methane, methanol, formate, and carbon dioxide, represent promising alternative feedstocks for sustainable biomanufacturing. This comprehensive review systematically analyzes the molecular mechanisms underlying microbial assimilation of C1 compounds, focusing on key metabolic pathways including the ribulose monophosphate (RuMP) pathway, xylulose monophosphate (XuMP) pathway, serine cycle, and the reductive glycine (rGly) pathway. We discuss recent advances in synthetic biology approaches for engineering C1-utilizing microorganisms, including pathway optimization, enzyme engineering, adaptive laboratory evolution, and compartmentalization strategies. Furthermore, we present an analysis of the development of the synthetic biology industry in major Chinese provinces and autonomous regions, including Xinjiang, Gansu, Ningxia, Hunan, and Guangdong. The review highlights the challenges and future directions in developing efficient C1-based cell factories for industrial applications, emphasizing the integration of multi-omics approaches, artificial intelligence, and systems metabolic engineering to enable next-generation C1 biotransformation platforms.

Keywords: one-carbon compounds, methylotrophy, synthetic biology, metabolic engineering, carbon fixation

Análise das vias de assimilação microbiana de compostos de um carbono e dos avanços na modificação por biologia sintética

Resumo

Compostos de um átomo de carbono (C1), como metano, metanol, formato e dióxido de carbono, representam matérias-primas promissoras para a biomanufatura sustentável. Esta revisão abrangente analisa sistematicamente os mecanismos moleculares subjacentes à assimilação microbiana de compostos C1, com foco nas principais vias metabólicas, incluindo a via do monofosfato de ribulose (RuMP), a via do monofosfato de xilulose (XuMP), o ciclo da serina e a via da glicina redutiva (rGly). Discutimos avanços recentes em abordagens de biologia sintética para a engenharia de microrganismos capazes de utilizar compostos C1, incluindo otimização de vias metabólicas, engenharia enzimática, evolução adaptativa em laboratório e estratégias de compartimentalização. Além disso, apresentamos uma análise do desenvolvimento da indústria de biologia sintética em importantes províncias e regiões autônomas da China, incluindo Xinjiang, Gansu, Ningxia, Hunan e Guangdong. A revisão destaca os desafios e as perspectivas futuras no desenvolvimento de fábricas celulares eficientes baseadas em C1 para aplicações industriais, enfatizando a integração de abordagens multi-ômicas, inteligência artificial e engenharia metabólica de sistemas para viabilizar plataformas de biotransformação de C1 de próxima geração.

Palavras-chave: compostos de um carbono, metilotrofia, biologia sintética, engenharia metabólica, fixação de carbono

1. Introduction

The global carbon cycle is fundamentally driven by microbial metabolism of one-carbon (C1) compounds, which plays a critical role in Earth's biogeochemical processes (Zhang et al., 2020). C1 compounds, including methane (CH_4), methanol (CH_3OH), formate ($HCOO^-$), carbon monoxide (CO), and carbon dioxide (CO_2), represent the simplest forms of carbon-containing molecules and serve as essential intermediates in the global carbon flux (Anthony, 1982; Hanson; Hanson, 1996).

In Figure **1**, we quantitatively depict the historical evolution, current scale, and forward-looking projections of the global synthetic biology market from 2018 to 2028, with authoritative data sourced from Strategic Market Research 2024, providing a data-driven panoramic overview of the industry's rapid expansion trajectory and future growth potential. It reveals a robust and sustained high-growth trend over the historical period, with the global synthetic biology market size surging from $5.3 billion in 2018 to an estimated $21 billion in 2024, representing a nearly 4-fold increase within 6 years.

This explosive expansion is driven by core technological breakthroughs in synthetic biology, growing global policy support, expanding downstream applications in biomanufacturing, pharmaceuticals, and sustainable agriculture, and surging capital investment in the global bioeconomy. The figure further projects that the market will maintain a strong growth momentum, with the size expected to reach $50 billion by 2028, corresponding to a compound annual growth rate of approximately 24.2% from 2024 to 2028. This quantitative analysis clarifies the strong market vitality of synthetic biology, providing a critical reference for academic research transformation, industrial investment layout, and policy formulation in this field.

In recent years, the utilization of C1 compounds as alternative feedstocks for biotechnological applications has gained significant attention due to several compelling factors (Yishai et al., 2018). First, C1 compounds can be produced from abundant and inexpensive sources, including natural gas, syngas, industrial waste gases, and captured CO_2, making them economically attractive alternatives to traditional sugar-based feedstocks. Second, the use of C1 compounds for biomanufacturing does not compete with food production, addressing the growing concern about the food-versus-fuel dilemma associated with first-generation biofuels (Meyer et al., 2018). Third, microbial conversion of C1 compounds, particularly CO_2 and CH_4, offers a promising approach for carbon capture and utilization (CCU), contributing to climate change mitigation efforts (Yang et al., 2024).

Methylotrophic microorganisms, which can utilize reduced C1 compounds as their sole carbon and energy source, have evolved sophisticated metabolic networks to assimilate these simple molecules into central metabolism (Chistoserdova et al., 2009). The key enzymes involved in C1 metabolism, including methane monooxygenase (MMO), methanol dehydrogenase (MDH), and various carbon fixation enzymes, catalyze the transformation of C1 substrates into metabolic intermediates that can be channeled into biosynthetic pathways (Anthony, 2004; Schmid et al., 2010).

The emergence of synthetic biology has revolutionized our ability to engineer microorganisms for enhanced C1 utilization (Paddon; Keasling, 2014). Through metabolic engineering, systems biology, and directed evolution, researchers have successfully constructed synthetic methylotrophs that efficiently convert C1 compounds into valuable products, including biofuels, chemicals, and pharmaceuticals (Bennett et al., 2018; Amir et al., 2025; Whitaker et al., 2015).

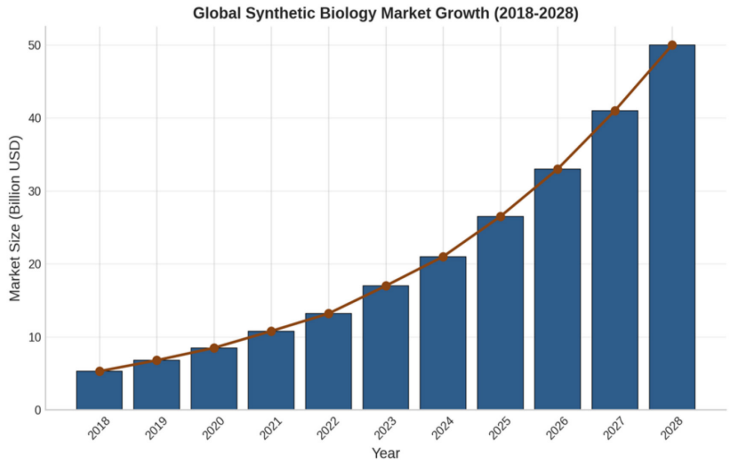


Figure 1. Global synthetic biology market growth from 2018 to 2028. The market size has increased from $5.3 billion in 2018 to an estimated $21 billion in 2024, with projections reaching $50 billion by 2028. Data source: Strategic Market Research, 2024.

China has emerged as a global leader in synthetic biology research and industrial applications, with significant investments in bio-manufacturing infrastructure and technology development. The synthetic biology industry in China has experienced rapid growth, with major development centers established in Guangdong, Shanghai, Beijing, and other provinces (see Figure 3 for regional distribution).

This review provides a comprehensive analysis of the molecular mechanisms underlying microbial C1 assimilation, focusing on the key metabolic pathways and enzymes involved. We discuss recent advances in synthetic biology approaches for engineering C1-utilizing microorganisms and present an analysis of the development of the synthetic biology industry in major Chinese provinces and autonomous regions. Finally, we highlight the challenges and future perspectives in developing efficient C1-based cell factories for sustainable biomanufacturing.

2. Molecular Mechanisms of C1 Compound Metabolism

2.1 Methane oxidation and methanol conversion

The aerobic oxidation of methane to methanol represents the first and rate-limiting step in methanotrophic metabolism (Dalton, 1992). This reaction is catalyzed by methane monooxygenase (MMO), which exists in two distinct forms: a membrane-bound particulate methane monooxygenase (pMMO) and a soluble cytoplasmic methane monooxygenase (sMMO) (Semrau et al., 1995; Stainthorpe et al., 1990).

The pMMO is a copper-containing enzyme encoded by the pmoCAB operon, consisting of three subunits (alpha, beta, and gamma) that form a trimeric complex embedded in the intracytoplasmic membranes (Lipscomb, 1994). The pMMO exhibits a higher affinity for methane (Km approximately 1-10 μM) than sMMO and is the predominant form found in methanotrophic bacteria growing at atmospheric methane concentrations (Dalton et al., 1985). The catalytic mechanism involves the activation of dioxygen at a dinuclear copper center, followed by the insertion of one oxygen atom into the C-H bond of methane.

The sMMO, encoded by the mmoXYBZDC operon, contains a diiron active center and is expressed under copper-limiting conditions (Murrell et al., 2000). While sMMO has broader substrate specificity and can oxidize a wider range of hydrocarbons, its lower affinity for methane makes it less efficient at oxidizing methane at ambient concentrations (Dalton, 2005).

Following methane oxidation, methanol is further converted to formaldehyde by methanol dehydrogenase (MDH). In Gram-negative methylotrophic bacteria, MDH is a pyrroloquinoline quinone (PQQ)-dependent enzyme located in the periplasm (Anthony, 2004). The enzyme is a heterotetramer (alpha2beta2), with the large alpha subunit (MxaF) containing the catalytic site and the small beta subunit (MxaI) serving a structural role. The reaction mechanism involves the oxidation of methanol to formaldehyde, with the concomitant reduction of PQQ to PQQH2, which is then reoxidized by cytochrome cL (Anthony, 1993).

2.2 Formaldehyde detoxification and assimilation

Formaldehyde represents a central metabolic intermediate in C1 metabolism but is also highly toxic to cells due to its reactivity with proteins and nucleic acids (Yurimoto et al., 2005). Methylotrophic microorganisms have evolved multiple strategies to manage formaldehyde toxicity while efficiently channeling it into biosynthetic pathways.

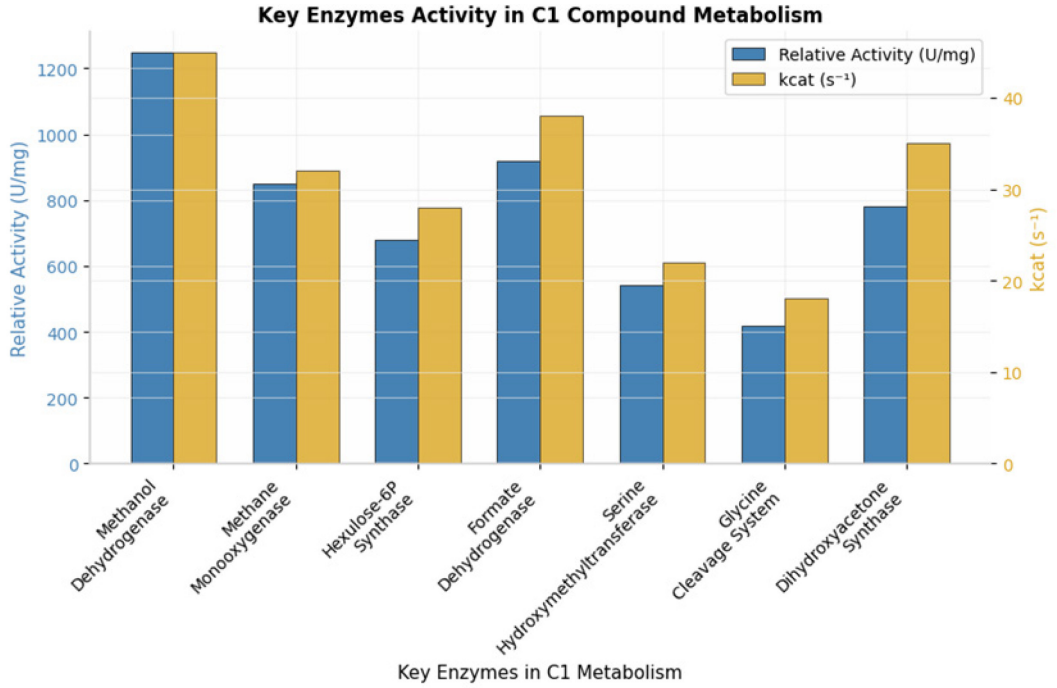
The primary detoxification mechanism involves the rapid capture of formaldehyde by various cofactors, including glutathione, mycothiol, tetrahydrofolate (THF), and tetrahydromethanopterin (H4MPT) (Muturi et al., 2017). The H4MPT-dependent pathway, widely distributed among methylotrophic bacteria, involves a series of enzymatic reactions that convert formaldehyde to formate via methylene-H4MPT and methenyl-H4MPT intermediates (Vorholt et al., 1999). Key enzymes in this pathway include methylene-H4MPT dehydrogenase (MtdB), methenyl-H4MPT cyclohydrolase (Mch), and formyltransferase/hydrolase complex (Fhc) (Chistoserdova et al., 1998).

In methylotrophic yeasts such as *Komagataella phaffii* (formerly *Pichia pastoris*), formaldehyde metabolism is compartmentalized within peroxisomes, where methanol oxidation by alcohol oxidase (AOX) generates formaldehyde and hydrogen peroxide (Gellissen, 2000). The toxic formaldehyde is immediately captured by xylulose-5-phosphate through the action of dihydroxyacetone synthase (DAS), initiating the XuMP pathway (Lindley,1981). The peroxisomal compartmentalization effectively isolates toxic intermediates from the cytosol, protecting cellular components from damage.

In Figure 2, we systematically quantify and compare the catalytic performance of core key enzymes involved in

microbial one-carbon (C1) compound assimilation metabolism, with relative enzyme activity (U/mg^{-1}) and catalytic turnover number (kcat, s^{-1}) as two core quantitative evaluation indicators, providing a critical benchmark for rate-limiting target identification and enzyme engineering optimization in C1 biotransformation. The selected enzymes cover key rate-limiting nodes of four mainstream C1 assimilation pathways: methane monooxygenase (MMO) and methanol dehydrogenase (MDH) responsible for upstream activation of methane and methanol; hexulose-6-phosphate synthase (HPS), the core enzyme of the ribulose monophosphate (RuMP) pathway; dihydroxyacetone synthase (DAS) for the xylulose monophosphate (XuMP) pathway; formate dehydrogenase (FDH) governing formate metabolism; and serine hydroxymethyltransferase (SHMT) and glycine cleavage system (GCS) for the serine cycle and reductive glycine pathway. This comparison clearly reveals the intrinsic catalytic capacity differences of enzymes at distinct C1 metabolic nodes, identifies the ubiquitous efficiency bottleneck of upstream C1 activation enzymes, and provides a core experimental reference for metabolic pathway optimization and efficient C1-utilizing cell factory construction.

Figure 2. Activity comparison of key enzymes involved in C1 compound metabolism. Relative enzyme activity



(U/mg^{-1}) and catalytic turnover number (kcat, s^{-1}) are shown for major enzymes in C1 assimilation pathways. MDH: methanol dehydrogenase; MMO: methane monooxygenase; HPS: hexulose-6-phosphate synthase; FDH: formate dehydrogenase; SHMT: serine hydroxymethyltransferase; GCS: glycine cleavage system; DAS: dihydroxyacetone synthase. Source: Authors, 2026.

2.3 Formate and CO_2 assimilation

Formate represents an important entry point for C1 assimilation, as it can be readily generated from various C1 compounds and electrochemically reduced from CO_2 (Bar-Even et al., 2010). The assimilation of formate into central metabolism occurs primarily through the serine cycle and the reductive glycine (rGly) pathway (Schrader et al., 2009).

The serine cycle, found in type II methanotrophs such as Methylobacterium extorquens, involves the condensation of methylene-THF with glycine to form serine, catalyzed by serine hydroxymethyltransferase (SHMT) (Peyraud et al., 2009). Serine is subsequently converted to phosphoenolpyruvate (PEP) and then to pyruvate, with the concomitant assimilation of CO_2 via PEP carboxylase (Peyraud et al., 2011). Glycine regeneration from acetyl-CoA occurs via the ethylmalonyl-CoA pathway, a complex series of reactions involving multiple enzymes (Erb et al., 2007).

The reductive glycine pathway, initially proposed as a theoretical synthetic route (Bar-Even et al., 2010), has recently been demonstrated in engineered *Escherichia coli* and *Saccharomyces cerevisiae* (Wu et al., 2012; Yishai et al., 2017). This pathway combines the THF-mediated conversion of formate to methylene-THF with the glycine cleavage system (GCS), which operates in reverse to condense methylene-THF, CO_2, and ammonia to form

glycine. The rGly pathway offers several advantages, including high ATP efficiency and minimal overlap with endogenous metabolism, making it an attractive target for metabolic engineering (Zarzycki et al., 2009).

3. Bioassimilation pathways for C1 compounds

3.1 Ribulose monophosphate (RuMP) pathway

The RuMP pathway represents the most energy-efficient route for formaldehyde assimilation and is widely distributed among methylotrophic bacteria (Anthony, 1982). The pathway consists of three main phases: fixation, cleavage, and rearrangement (Large et al., 1961; Strom et al., 1974).

In the fixation phase, formaldehyde condenses with ribulose-5-phosphate (Ru5P) to form hexulose-6-phosphate (H6P), catalyzed by hexulose-6-phosphate synthase (HPS) (Ferenci et al., 1974). H6P is subsequently isomerized to fructose-6-phosphate (F6P) by hexulose-6-phosphate isomerase (HPI) (Lindley, 1981). The cleavage phase involves the conversion of F6P to glyceraldehyde-3-phosphate (GAP) and either dihydroxyacetone phosphate (DHAP) or pyruvate, depending on the aldolase variant present (Hou, 1981). The rearrangement phase regenerates Ru5P through a series of transketolase and transaldolase reactions, completing the cycle (Wright; Alefounder, 1991).

The RuMP pathway offers significant advantages for biotechnological applications due to its high carbon efficiency and ATP yield. For every three molecules of formaldehyde assimilated, one molecule of pyruvate is produced with a net gain of one ATP and one NADH (Bennett et al., 2018). This energy efficiency makes the RuMP pathway a preferred target for engineering synthetic methylotrophy in industrial hosts such as *E. coli* and *Corynebacterium glutamicum* (Muller et al., 2015; Witthoff et al., 2015).

3.2 Xylulose monophosphate (XuMP) pathway

The XuMP pathway, also known as the dihydroxyacetone (DHA) cycle, is the primary route for methanol assimilation in methylotrophic yeasts (Lindley, 1981). The pathway is compartmentalized within peroxisomes, where methanol oxidation by alcohol oxidase (AOX) generates formaldehyde, which is immediately captured by xylulose-5-phosphate (Xu5P) through the action of dihydroxyacetone synthase (DAS) (Douma et al., 1985).

The DAS-catalyzed reaction produces dihydroxyacetone (DHA) and glyceraldehyde-3-phosphate (GAP), which are subsequently phosphorylated and enter gluconeogenesis (Mayer et al., 1995). The carbon rearrangement reactions regenerate Xu5P through a series of reactions involving transketolase, transaldolase, and various isomerases (RuBmayer et al., 2015). The XuMP pathway, like the RuMP pathway, assimilates three molecules of formaldehyde to produce one molecule of GAP (Gellissen, 2000).

The peroxisomal compartmentalization of the XuMP pathway provides an effective strategy for managing formaldehyde toxicity, as the toxic intermediate is generated and immediately consumed within the same organelle. This spatial organization has inspired engineering strategies to establish synthetic methylotrophy in non-native hosts, including targeting heterologous enzymes to peroxisomes in *S. cerevisiae* (Heux et al., 2015).

In Figure 3, we systematically quantify and compare the core metabolic performance of six mainstream microbial one-carbon (C1) compound assimilation pathways, with ATP consumption (mol/mol C1) and theoretical carbon yield (%) as two core evaluation indicators that determine the atom economy, energy efficiency, and industrial application potential of C1-based biomanufacturing. The evaluated pathways cover the dominant natural C1 assimilation routes in aerobic and anaerobic microorganisms: ribulose monophosphate (RuMP), xylulose monophosphate (XuMP), serine cycle, reductive glycine (rGly), ribulose bisphosphate (RuBP), and Wood-Ljungdahl (WL) pathways. The comparison clearly reveals significant performance differences across pathways: the WL pathway exhibits the highest theoretical carbon yield (near 100%) and the lowest ATP demand, while RuMP and XuMP pathways show excellent low energy consumption and high carbon yield for aerobic methanol utilization.

In contrast, the RuBP pathway has the highest ATP consumption and relatively low carbon yield, and the rGly pathway stands out for its balanced energy efficiency and broad host compatibility. This quantitative benchmark provides a core theoretical reference for pathway selection, metabolic engineering optimization, and the design of synthetic pathways for efficient C1-utilizing microbial cell factories.

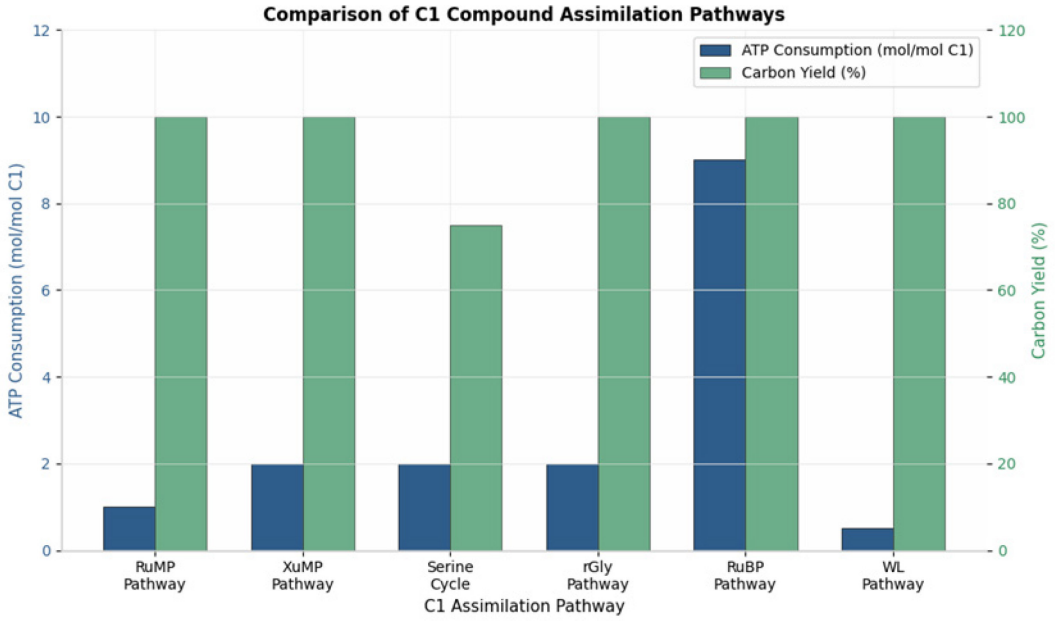


Figure 3. Comparison of C1 compound assimilation pathways. ATP consumption (mol/mol C1) and theoretical carbon yield (%) are compared for major C1 assimilation pathways: RuMP (ribulose monophosphate), XuMP (xylulose monophosphate), serine cycle, rGly (reductive glycine), RuBP (ribulose bisphosphate), and WL (Wood-Ljungdahl) pathways. Source: Authors, 2026.

3.3 Serine cycle and related pathways

The serine cycle is the primary C1 assimilation pathway in type II methanotrophs and many facultative methylotrophs (Peyraud et al., 2009). Unlike the RuMP and XuMP pathways, which assimilate formaldehyde directly, the serine cycle utilizes formate as the C1 substrate, which is first converted to methylene-THF through a series of THF-dependent reactions (Chistoserdova et al., 2003).

The key enzyme of the serine cycle is serine hydroxymethyltransferase (SHMT), which catalyzes the condensation of methylene-THF with glycine to form serine (Wilson et al., 1993). Serine is subsequently deaminated to hydroxypyruvate and reduced to glycerate, which is then phosphorylated and converted to 2-phosphoglycerate, entering central metabolism. The regeneration of glycine from acetyl-CoA occurs through the ethylmalonyl-CoA pathway, involving a complex series of reactions including crotonyl-CoA carboxylase/reductase (CCR) and ethylmalonyl-CoA mutase (Erb et al., 2007, 2009).

The serine cycle has lower energy efficiency compared to the RuMP pathway, requiring two molecules of ATP and NADH per molecule of pyruvate produced (Zarzycki et al., 2009). However, the serine cycle offers the advantage of simultaneous assimilation of formate and CO_2, making it suitable for autotrophic growth conditions (Kalyuzhnaya et al., 2013).

3.4 Reductive glycine pathway

The reductive glycine (rGly) pathway represents a recently discovered linear pathway for formate assimilation that combines features of the serine cycle and the Wood-Ljungdahl pathway (Bar-Even et al., 2010). The pathway was initially proposed through computational analysis as a potentially optimal route for aerobic C1 assimilation and has since been experimentally demonstrated in engineered microorganisms.

The core module of the rGly pathway involves the conversion of formate to methylene-THF through THF-dependent reactions, followed by the condensation of methylene-THF with CO_2 and ammonia to form glycine, catalyzed by the glycine cleavage system (GCS) operating in reverse (Yishai et al., 2017). Glycine can then be converted to serine via SHMT or directly to pyruvate under anaerobic conditions (Wu et al., 2012).

The rGly pathway offers several advantages for metabolic engineering applications. First, it requires only two ATP molecules per molecule of serine produced, comparable to the RuMP pathway in terms of energy efficiency (Bar-Even et al., 2010). Second, the pathway has minimal overlap with endogenous metabolism, reducing the potential

for metabolic interference. Third, most of the required enzymes are ubiquitously distributed among microorganisms, facilitating heterologous expression (Zarzycki et al., 2009). Recent studies have successfully implemented the rGly pathway in *E. coli*, enabling growth on formate as the sole carbon source (Wu et al., 2012; Keller et al., 2022).

4. Synthetic Biology Modification Research Progress

4.1 Engineering synthetic methylotrophs

The engineering of synthetic methylotrophs in non-native hosts has emerged as a major research focus in synthetic biology, driven by the potential to leverage well-characterized industrial platforms for C1-based biomanufacturing (Antoniewicz, 2019). Key host organisms include *E. coli*, *S. cerevisiae*, *Corynebacterium glutamicum*, and *Yarrowia lipolytica*, each offering distinct advantages in terms of genetic tools, industrial scalability, and product range (Bae et al., 2022; Zeng et al., 2025).

The first demonstration of synthetic methylotrophy in *E. coli* was achieved by expressing the RuMP pathway genes from *Bacillus methanolicus*, enabling methanol-dependent growth on glucose (Muller et al., 2015). Subsequent studies improved methanol assimilation efficiency by optimizing gene expression, codon usage, and cofactor balance (Bennett et al., 2018). The integration of adaptive laboratory evolution (ALE) strategies has further enhanced methanol utilization, with evolved strains showing significantly improved growth rates and methanol consumption (Wang et al., 2019).

In *S. cerevisiae*, synthetic methylotrophy has been achieved by heterologously expressing the XuMP pathway genes from K. phaffii, combined with peroxisomal targeting strategies to compartmentalize methanol oxidation (Dai et al., 2017). Recent advances have demonstrated methanol-dependent growth through the integration of evolutionary engineering approaches, although robust growth on methanol as the sole carbon source remains challenging (Zhan et al., 2023).

In Figure 4, we systematically map the decade-long developmental trajectory of synthetic methylotrophs spanning 2015 to 2024, quantify the progressive reduction in doubling time (hours) of engineered strains driven by metabolic engineering and adaptive laboratory evolution (ALE), and mark the landmark breakthroughs shaping the field. It reveals a continuous improvement in growth performance of synthetic methylotrophic chassis: the doubling time of engineered strains decreases stepwise through iterative pathway optimization, key enzyme engineering, and ALE-mediated adaptive remodeling, achieving a leap from initial C1 assimilation verification to robust methanol-dependent growth.

Key milestone events are chronologically highlighted: the first 13C-methanol incorporation in *E. coli* in 2015, providing the first proof-of-concept for heterologous methanol assimilation in model non-methylotrophs; the establishment of methanol-dependent growth in *E. coli* in 2020, a critical breakthrough realizing synthetic methylotrophy with methanol as the sole carbon source; and evolutionary engineered *Saccharomyces cerevisiae* in 2024, expanding synthetic methylotrophy to eukaryotic chassis. This timeline clarifies the iterative development logic of synthetic methylotrophs, providing a critical reference for constructing next-generation efficient C1-utilizing cell factories.

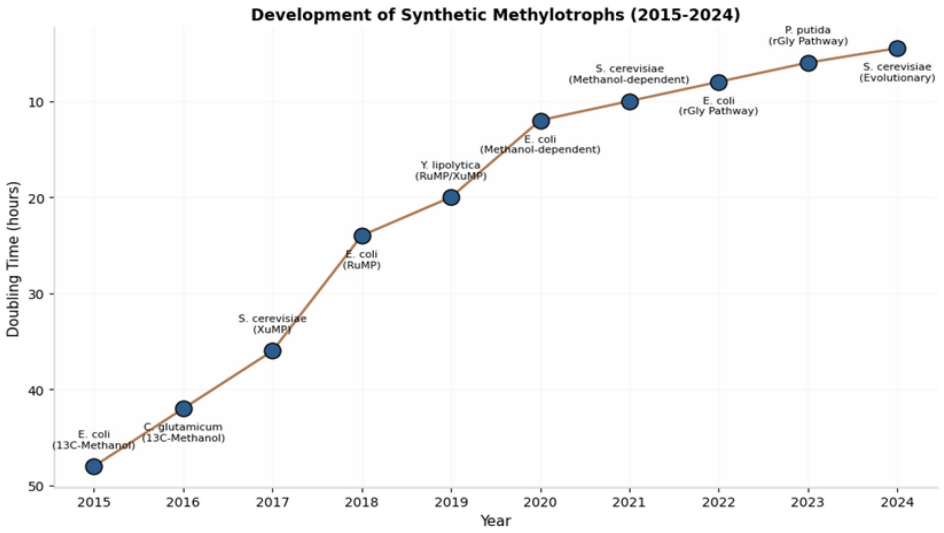


Figure 4. Development timeline of synthetic methylotrophs (2015-2024). The doubling time (hours) of engineered

strains has progressively decreased through metabolic engineering and adaptive laboratory evolution. Key milestones include the first 13C-methanol incorporation in *Escherichia coli* (2015), methanol-dependent growth in *Escherichia coli* (2020), and evolutionary engineered *Saccharomyces cerevisiae* (2024). Source: Authors, 2026.

4.2 Pathway optimization strategies

The optimization of C1 assimilation pathways involves multiple strategies targeting enzyme engineering, flux balance, and metabolic integration (Cotton et al., 2020). Computational tools such as flux balance analysis (FBA) and kinetic modeling have been employed to identify rate-limiting steps and predict optimal pathway configurations (Noor et al., 2014;Beste et al., 2011).

Enzyme engineering approaches have focused on improving the catalytic efficiency and substrate specificity of key enzymes in C1 metabolism. For methanol dehydrogenase, directed evolution has generated variants with enhanced activity and improved cofactor affinity (Park et al., 2017). Similarly, hexulose-6-phosphate synthase (HPS) has been engineered for increased thermostability and catalytic turnover, addressing one of the major bottlenecks in the RuMP pathway (Ochsner et al., 2015).

The balance of cofactor supply, particularly NADH and ATP, is critical for efficient C1 assimilation (Heux et al., 2015). Strategies to enhance cofactor availability include the engineering of NADH regeneration systems, optimization of electron transport chains, and the introduction of non-canonical redox cofactors (Bogorad et al., 2013). Recent studies have demonstrated that fine-tuning the $NADH/NAD^+$ ratio can significantly improve methanol assimilation efficiency in engineered strains.

4.3 Adaptive laboratory evolution

Adaptive laboratory evolution (ALE) has proven to be a powerful approach for improving C1 utilization in engineered strains, particularly for complex phenotypes that are difficult to predict or engineer rationally (Dragosits; Mattanovich, 2013). By subjecting populations to continuous selection under methanol-limiting conditions, researchers have isolated evolved strains with significantly improved growth rates and methanol consumption.

Genomic analysis of evolved strains has revealed mutations in diverse cellular processes, including carbon metabolism, stress response, and membrane transport (Tuyishime et al., 2018). In *E. coli* engineered for methanol utilization, ALE-selected mutations frequently occur in genes related to formaldehyde detoxification, methanol oxidation, and central carbon metabolism (Bennett et al., 2020). These findings have provided valuable insights into the genetic determinants of efficient methylotrophy and guided rational engineering strategies.

The combination of ALE with genome-wide screening approaches, such as CRISPR-Cas9-mediated genome editing, has accelerated the identification of beneficial mutations and their subsequent integration into engineered strains (Jakociunas et al., 2015). This integrated approach has been successfully applied to improve methanol tolerance and utilization in multiple host organisms, including *C. glutamicum* and *Y. lipolytica* .

In Figure **5**, we quantitatively characterize the application frequency and research focus distribution of mainstream metabolic engineering strategies for microbial one-carbon (C1) compound utilization from 2020 to 2024, providing a panoramic data-driven reference for the rational design of efficient C1-utilizing microbial cell factories. Statistical results reveal a clear concentration of research hotspots: pathway optimization (28%) and enzyme engineering (22%) are the most widely adopted strategies, which mainly address core bottlenecks of C1 assimilation, including rate-limiting steps of metabolic pathways, thermodynamic barriers, and low catalytic activity of key C1-activating enzymes. Adaptive laboratory evolution (18%) ranks third, serving as a critical tool to improve the compatibility between heterologous C1 pathways and host chassis. Cofactor engineering (12%), subcellular compartmentalization (10%), and precision genome editing (10%) are applied as complementary strategies to optimize energy/reducing power balance, enhance intermediate metabolite enrichment, and remodel the host metabolic network. This distribution clarifies current technical preferences and development trends in C1 metabolic engineering, guiding the optimization of subsequent engineering design strategies.

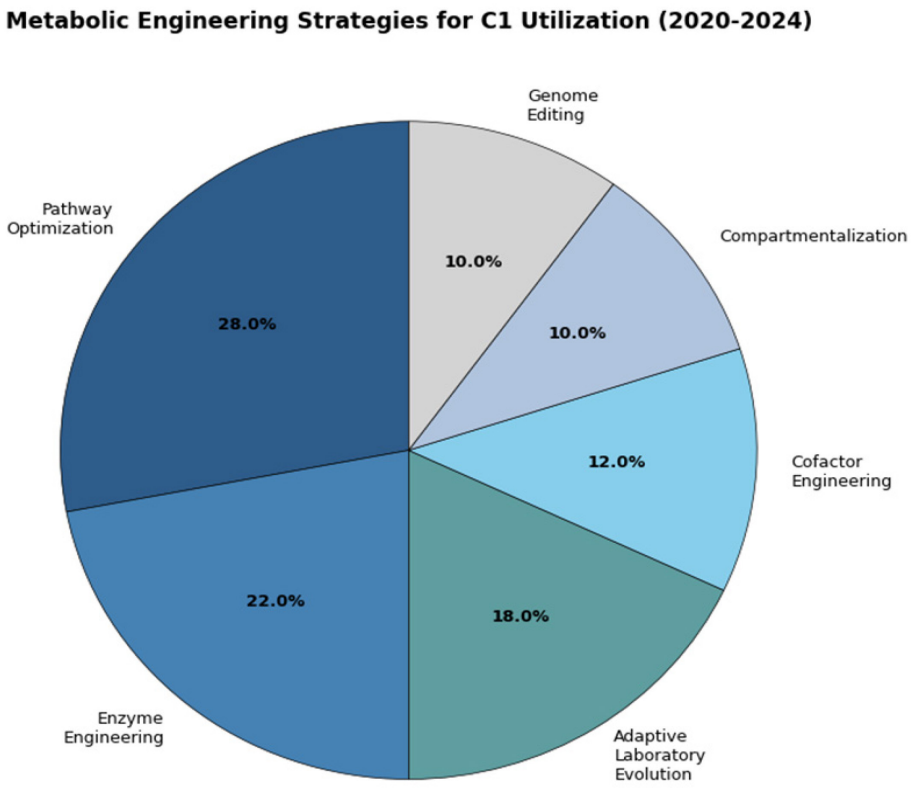


Figure 5. Distribution of metabolic engineering strategies for C1 utilization (2020-2024). Pathway optimization (28%) and enzyme engineering (22%) represent the most frequently applied strategies, followed by adaptive laboratory evolution (18%), cofactor engineering (12%), compartmentalization (10%), and genome editing (10%). Source: Authors, 2026.

4.4 Product biosynthesis from C1 compounds

Engineered methylotrophs have been successfully developed for the production of a wide range of valuable products, including biofuels, chemicals, and pharmaceuticals (Haynes; Gonzalez, 2014). The direct conversion of C1 compounds to target products offers significant advantages in terms of carbon efficiency and process economics compared to traditional sugar-based fermentation (Bogorad et al., 2013).

In the biofuels sector, engineered methanotrophs have demonstrated production of fatty acid methyl esters (FAMEs) with titers exceeding 111 mg/g^{-1} dry cell weight in *Methylomicrobium buryatense* (Demidenko et al., 2017). Further optimization through high-cell-density cultivation achieved lipid productivities of 45.4 mg/L/h^{-1}, representing a threefold improvement over previous report. These advances highlight the potential of methanotrophic bacteria for sustainable biofuel production from methane.

The production of platform chemicals from methanol has been extensively explored in engineered methylotrophic yeasts and bacteria. In *K. phaffii*, metabolic engineering of the XuMP pathway and optimization of methanol induction strategies have enabled high-level production of recombinant proteins, with titers exceeding 10 g/L^{-1} for various pharmaceutical products (Gasser et al., 2013). For small molecule production, engineered strains have achieved significant improvements in the synthesis of organic acids, amino acids, and terpenoids (Celinska, 2017).

In Figure 6, we have systematically quantified the product synthesis performance (titer and yield) of metabolically engineered microbial strains using one-carbon (C1) compounds as sole or co-feedstock from 2020 to 2024, comprehensively demonstrating the product spectrum expansion and synthetic efficiency breakthroughs of C1-based sustainable biomanufacturing. It covers the dominant C1-derived product categories: high-value fine chemical mevalonate (mg/L^{-1} scale), and bulk bio-based chemicals including fatty acids, lactic acid, isobutanol, glycolate, succinate, and key platform compound 3-hydroxypropionate (3-HP, all at g/L^{-1} scale), spanning applications in biofuels, polymer monomers, and pharmaceutical intermediates. The figure clearly reveals that significant improvements in product titers have been achieved through continuous iterative metabolic engineering

efforts, breaking the compatibility bottleneck between heterologous C1 assimilation pathways and product synthesis modules. This quantitative overview provides a critical performance benchmark for constructing next-generation efficient C1-utilizing cell factories, and fully verifies the industrialization potential of C1 compounds as alternative feedstocks for sugar-based biomanufacturing.

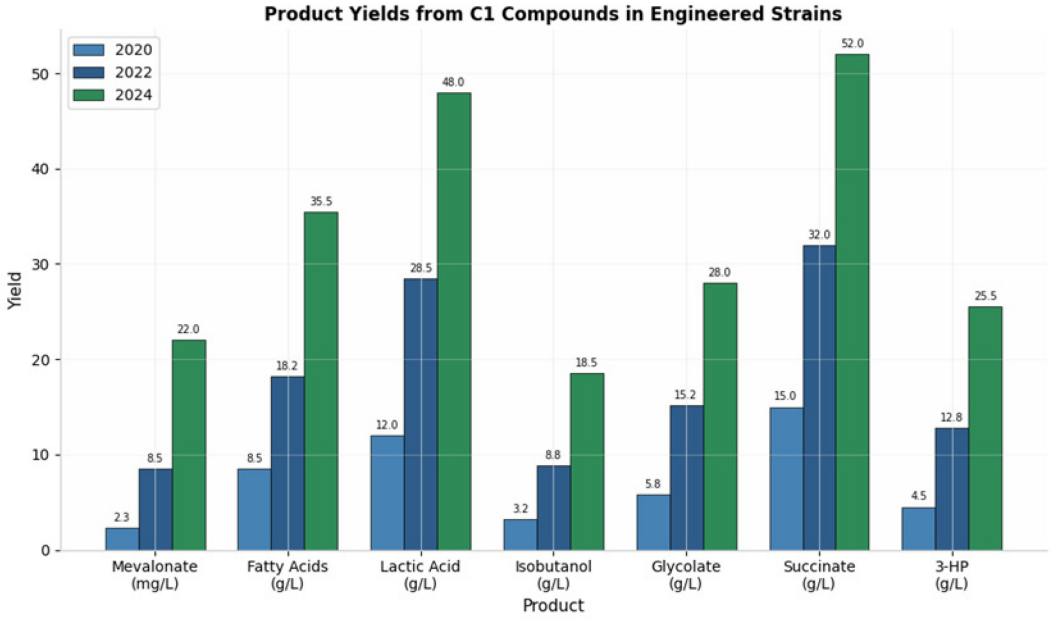


Figure 6. Product yields from C1 compounds in engineered strains (2020-2024). Significant improvements in product titers have been achieved through continuous metabolic engineering efforts. Mevalonate (mg/L^{-1}), fatty acids (g/L^{-1}), lactic acid (g/L^{-1}), isobutanol (g/L^{-1}), glycolate (g/L^{-1}), succinate (g/L^{-1}), and 3-HP (3-hydroxypropionate, g/L^{-1}) represent major product categories. Source: Authors, 2026.

Recent advances in the production of mevalonate from methanol have achieved titers of 2.27 g/L^{-1} in engineered *Methylorubrum extorquens* following adaptive laboratory evolution. Similarly, the production of 2-hydroxyisobutyrate and 3-hydroxyisobutyrate from methanol has been demonstrated in engineered C. glutamicum strains, with yields approaching 50% of the theoretical maximum. These achievements demonstrate the growing potential of C1-based biomanufacturing for industrial applications.

5. Other thinking, Synthetic Biology Industry Development in China

5.1 National overview

China has emerged as a global leader in synthetic biology research and industrial applications, with significant government support and private investment driving rapid growth in the sector. According to the China Synthetic Biology Industry White Paper 2024, the global synthetic biology market has grown from $5.3 billion in 2018 to over $17 billion in 2023, with an average annual growth rate of 27%, and is projected to approach $50 billion by 2028 (Strategic Market Research, 2024).

China's synthetic biology industry benefits from the country's world-leading fermentation infrastructure, with an annual output of fermentation products exceeding 30 million tons, accounting for approximately 70% of global fermentation capacity (Shenzhen Industrial Innovation Center, 2024). This manufacturing advantage provides a strong foundation for the industrialization of synthetic biology technologies, particularly for bulk bio-based products such as amino acids, organic acids, and vitamins.

Government policies have played a crucial role in promoting synthetic biology development at both national and regional levels. The 14th Five-Year Plan for Science and Technology Innovation explicitly supports synthetic biology research, while multiple provinces and municipalities have established dedicated funding programs and industrial parks for synthetic biology enterprises (China Ministry of Science and Technology, 2021).

In Figure 7, we have systematically depicted the spatial distribution pattern of China's synthetic biology industry at the provincial and regional level in 2024, with the number of core operating enterprises and total industry

revenue (billion RMB) as core quantitative evaluation indicators, providing a panoramic data-driven overview of the industry's development gradient, regional positioning, and growth potential. It clearly identifies that Guangdong, Shanghai, and Beijing form the first echelon of industrial development, leading the country in both enterprise count and total revenue, driven by their abundant high-end R&D resources, complete industrial chain supporting system, concentrated capital elements, and continuous policy support for the bioeconomy. Meanwhile, the figure highlights Xinjiang, Gansu, and Ningxia in northwest China as key emerging industrial regions with outstanding growth potential, which rely on rich low-cost one-carbon feedstock endowments and energy cost advantages to form a differentiated development path for bulk bio-based product manufacturing. This analysis clarifies the current development pattern of China's synthetic biology industry, providing a scientific reference for regional industrial layout optimization and coordinated development of the national bioeconomy.

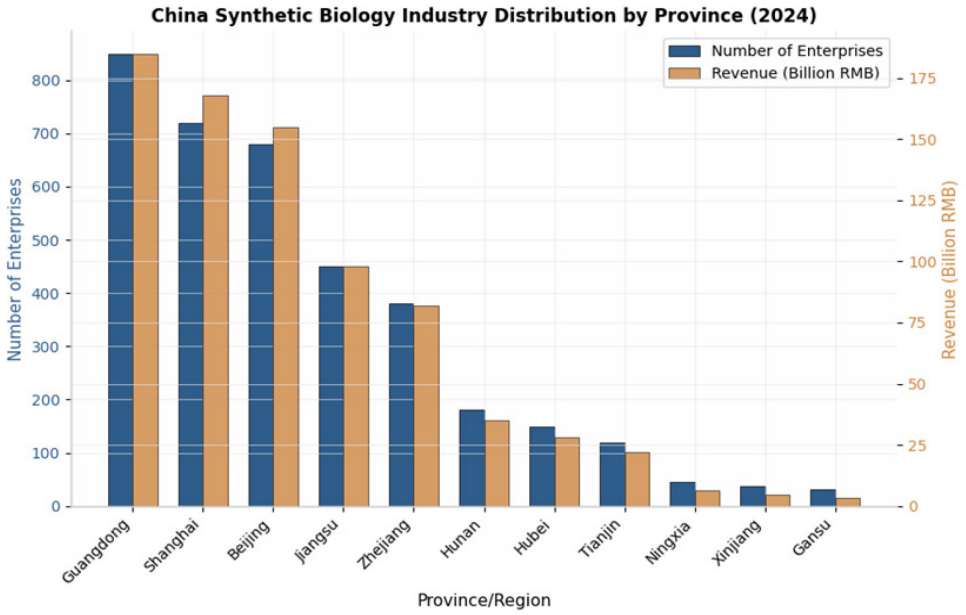


Figure 7. Distribution of China's synthetic biology industry by province/region (2024). The number of enterprises and revenue (billion RMB) are shown for major provinces and autonomous regions. Guangdong, Shanghai, and Beijing lead in both enterprise count and revenue, while Xinjiang, Gansu, and Ningxia represent emerging regions with significant growth potential. Source: Authors, 2026.

5.2 Regional development analysis

5.2.1 Guangdong Province

Guangdong Province stands at the forefront of China's synthetic biology industry, with over 850 enterprises generating approximately 185 billion RMB in revenue in 2024 (Guangdong Provincial Government, 2024). The province's leadership position is supported by its strong foundation in biotechnology, advanced manufacturing capabilities, and a favorable policy environment. The Guangzhou-Shenzhen Science and Technology Innovation Corridor hosts numerous synthetic biology research institutions and companies, forming a comprehensive innovation ecosystem.

The Shenzhen Industrial Innovation Center for Engineering Biology, established in collaboration with the Shenzhen Institutes of Advanced Technology (SIAT), represents China's first integrated innovation and entrepreneurship complex for synthetic biology (SIAT, 2024). The center's unique upstairs-downstairs model facilitates the translation of basic research into commercial applications, with researchers conducting fundamental science upstairs while downstream industrial incubation occurs downstairs.

5.2.2 Hunan Province

Hunan Province has emerged as an important hub for synthetic biology in central China, with approximately 180 enterprises and 35 billion RMB in revenue (Hunan Provincial Government, 2024). The province's strengths in biomedical research and traditional Chinese medicine provide unique opportunities for synthetic biology

applications in pharmaceutical development and natural product synthesis.

Key research institutions in Hunan, including Central South University and Hunan University, have established dedicated synthetic biology research centers focusing on metabolic engineering and natural product biosynthesis (Central South University, 2023). The province has also invested in industrial parks and incubators to support the commercialization of synthetic biology technologies.

5.2.3 Ningxia Hui Autonomous Region

Ningxia represents a rapidly developing region for synthetic biology in northwest China, with 45 enterprises generating 6.5 billion RMB in revenue (Ningxia Development and Reform Commission, 2024). The region's abundant coal and natural gas resources provide cost-competitive feedstocks for C1-based biomanufacturing, positioning Ningxia as a potential hub for methanol and syngas fermentation industries.

The Ningxia Energy and Chemical Industry Base has attracted significant investment in synthetic biology projects focused on converting coal-derived syngas to high-value chemicals (Ningxia Investment Promotion Bureau, 2023). Government incentives, including tax breaks and subsidized land use, have encouraged enterprises to establish production facilities in the region.

5.2.4 Xinjiang Uygur Autonomous Region

Xinjiang's synthetic biology industry, though still in early development stages, shows significant potential due to the region's abundant natural gas and renewable energy resources (Xinjiang Development and Reform Commission, 2024). With 38 enterprises and 4.8 billion RMB in revenue, the region is positioning itself as a center for methane-based biomanufacturing and biofuel production.

The Xinjiang Production and Construction Corps has established partnerships with leading research institutions to develop synthetic biology technologies for agricultural applications, including biofertilizers and biostimulants (XPCC, 2023). The region's vast land area and abundant sunlight also support the development of algae-based carbon capture and bioproduction systems.

5.2.5 Gansu Province

Gansu Province, with 32 synthetic biology enterprises and 3.2 billion RMB in revenue, is leveraging its strengths in traditional fermentation industries to develop modern synthetic biology capabilities (Gansu Provincial Government, 2024). The Lanzhou New Area has established a biotechnology industrial park focused on enzyme engineering and biocatalysis applications.

Research institutions in Gansu, including Lanzhou University, have developed expertise in extremophile microbiology, providing unique genetic resources for engineering stress-tolerant industrial strains (Lanzhou University, 2023). The province's strategic location along the Belt and Road Initiative corridors also facilitates international collaboration and technology transfer.

5.3 Industry challenges and opportunities

Despite rapid growth, China's synthetic biology industry faces several challenges that need to be addressed to maintain competitiveness. A critical issue is the low self-sufficiency rate for industrial microbial strains, with core production strains for antibiotics, vitamins, and amino acids largely controlled by foreign companies such as CJ and Ajinomoto (Guangzhou Science and Technology Bureau, 2024).

Intellectual property constraints and regulatory uncertainties also pose challenges for industry development. The current regulatory framework for genetically modified microorganisms in industrial applications requires further clarification to support innovation while ensuring biosafety (China Ministry of Agriculture, 2023). Additionally, the shortage of interdisciplinary talent combining biology, engineering, and computational skills limits the pace of technological advancement.

On the other hand, significant opportunities exist for continued growth and innovation. The integration of artificial intelligence and machine learning with synthetic biology is accelerating the design-build-test-learn cycle, enabling more efficient strain development (Koh, 2025). The growing demand for sustainable and bio-based products, driven by environmental regulations and consumer preferences, provides expanding market opportunities for synthetic biology applications.

6. Challenges and Future Perspectives

6.1 Current challenges

Despite significant advances in C1 compound microbial assimilation and synthetic biology modification, several challenges remain to be addressed for the widespread industrial application of C1-based biomanufacturing (Yang et al., 2024).

Low carbon fixation efficiency represents a major bottleneck in current C1 utilization systems. The theoretical maximum carbon yield is often not achieved due to metabolic inefficiencies, cofactor limitations, and competing pathways (Bar-Even et al., 2010). Improving the overall carbon conversion efficiency requires systems-level optimization of metabolic networks, including the elimination of byproduct formation and enhancement of precursor supply (Heux et al., 2015).

The toxicity of C1 intermediates, particularly formaldehyde, poses significant challenges for engineering efficient methylotrophs (Yurimoto et al., 2005). Even at low concentrations, formaldehyde can cause protein damage and DNA cross-linking, limiting the maximum methanol concentration that can be used in bioprocesses. Strategies to enhance formaldehyde tolerance include pathway compartmentalization, expression of detoxification enzymes, and evolutionary engineering.

Slow growth rates of synthetic methylotrophs compared to sugar-based fermentation remain a significant limitation for industrial applications (Muller et al., 2015). The lower energy efficiency of C1 metabolism and the metabolic burden of heterologous pathway expression contribute to reduced growth rates, affecting process productivity and economics (Witthoff et al., 2015).

6.2 Future perspectives

The integration of multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, provides unprecedented opportunities for systems-level understanding and optimization of C1 metabolism (Keller et al., 2022). Comprehensive analysis of methylotrophic organisms at multiple molecular levels can reveal novel regulatory mechanisms and identify targets for metabolic engineering (Chistoserdova, 2011).

Artificial intelligence and machine learning are increasingly being applied to synthetic biology, enabling predictive modeling of metabolic pathways, enzyme design, and strain optimization (Koh, 2025). AI-powered tools such as protein language models can significantly reduce the experimental screening burden for enzyme engineering, accelerating the development of improved biocatalysts for C1 utilization.

The development of cell-free synthetic biology systems offers an alternative approach for C1 compound utilization, bypassing the constraints of cellular metabolism and toxicity. Cell-free systems enable precise control over reaction conditions and cofactor supply, potentially achieving higher carbon conversion efficiencies than whole-cell systems (Karim; Jewett, 2016).

Novel C1 assimilation pathways discovered through computational design and metagenomic mining may provide alternative routes for efficient carbon fixation. The continued exploration of natural biodiversity and the rational design of synthetic pathways will expand the toolkit available for engineering C1-utilizing organisms.

7. Conclusions

The microbial assimilation of C1 compounds represents a promising approach for sustainable biomanufacturing, offering the potential to convert abundant and inexpensive feedstocks into valuable products while contributing to carbon emission reduction goals. Significant progress has been made in understanding the molecular mechanisms of C1 metabolism and in developing synthetic biology tools for engineering efficient C1-utilizing microorganisms.

The RuMP, XuMP, serine, and reductive glycine pathways each offer distinct advantages for C1 assimilation, and the choice of pathway depends on the specific application and host organism. Metabolic engineering strategies, including pathway optimization, enzyme engineering, and adaptive laboratory evolution, have enabled significant improvements in C1 utilization efficiency and product yields.

China's synthetic biology industry has experienced rapid growth, with major development centers in Guangdong, Shanghai, Beijing, and emerging regions including Xinjiang, Gansu, Ningxia, and Hunan. Continued investment in research infrastructure, talent development, and regulatory frameworks will be essential for maintaining competitiveness in the global synthetic biology market.

Future research should focus on addressing the remaining challenges of carbon fixation efficiency, intermediate toxicity, and growth rate limitations through the integration of systems biology, artificial intelligence, and advanced engineering strategies. The development of efficient C1-based cell factories will contribute to the

transition toward a sustainable bioeconomy, enabling the production of fuels, chemicals, and materials from renewable carbon sources.

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8. Authors' Contributions

Baoxin Zhang: conceptualization and data curation. *Hao Sun*: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, writing – review & editing. *Hailei Zhang*: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, supervision, validation, visualization. *Li Zhu*: methodology. *Xiang Weng*: investigation, methodology, and data analysis.

9. Conflicts of Interest

No conflicts of interest.

10. Ethics Approval

Not applicable.

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