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Review 综述

混合碳源协同代谢驱动高值化学品生物合成的研究进展

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ZHAO Quanlu, ZHANG Weiqiang, WU Zhuoheng, WANG Kai, CHEN Biqiang, TAN Tianwei. Research progress in high-value chemical biosynthesis driven by synergistic metabolism of mixed carbon sources[J]. Acta Microbiologica Sinica, 2026, 66(9): 4471-4495.

摘　要：在“碳中和”目标指引下，提升微生物细胞工厂的碳原子利用经济性已成为新时期绿色生物制造领域的核心科学问题与关键技术需求。传统发酵工艺虽具备成熟的工业应用基础，但受限于中心碳代谢途径中氧化脱羧反应导致的碳损失，原料碳利用效率难以实现根本性突破，制约了生物制造产业的绿色化与高质量发展。多碳源耦合发酵通过理性整合糖类有机底物与甲酸、甲醇、合成气等 C1 碳源及乙酸等 C2 碳源，构建碳架互补、能量协同、还原力平衡的混合营养代谢体系，为突破传统生物发酵的碳得率理论瓶颈提供技术路径。本文系统综述了合成生物学技术驱动下多碳源耦合发酵领域的研究进展，重点阐释有机底物与 C1/C2 化合物及工业尾气等典型耦合体系的代谢途径重构与调控机制，梳理了该技术在合成燃料、生物基材料和高附加值天然产物合成中的应用实例与研究现状，并讨论了其在碳捕获利用与生物制造高值化融合发展中的工业化前景，以期为面向碳中和的高效生物制造技术创新提供理论参考与思路借鉴。

关键词：多碳源耦合发酵；高值化学品；合成生物学；碳中和

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Research progress in high-value chemical biosynthesis driven by synergistic metabolism of mixed carbon sources

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Abstract: Under the strategic goal of carbon neutrality, enhancing the carbon atom utilization efficiency of microbial cell factories has emerged as a core scientific challenge and imperative technical demand for advanced green biomanufacturing. Conventional fermentation processes possess mature industrial applicability, yet they are constrained by inevitable carbon losses *via* oxidative decarboxylation in central carbon metabolism. Such inherent limitation hinders fundamental improvement in substrate carbon conversion efficiency and restricts the green and high-quality advancement of the biomanufacturing industry. Multi-carbon source co-fermentation enables the rational integration of carbohydrate substrates with C1 feedstocks (formic acid, methanol, and syngas) and C2 feedstocks (acetic acid), constructing a mixotrophic metabolic system featured with carbon skeleton complementation, energy supply synergy, and intracellular redox balance. This strategy offers an innovative technological paradigm to break the theoretical carbon yield bottleneck of conventional bioprocesses. This paper systematically reviews the advances in multi-carbon source co-fermentation driven by synthetic biology. We emphatically elaborate on metabolic pathway reconstruction and regulatory mechanisms of typical co-fermentation systems consisting of organic substrates, C1/C2 compounds, and industrial waste gas. The state-of-the-art applications in synthetic fuels, bio-based materials, and high-value natural product biosynthesis are summarized. Furthermore, this paper discusses the industrial potential of this technology in integrating carbon capture and utilization and high-value biomanufacturing, aiming to provide a theoretical basis and strategic references for the innovation of efficient biomanufacturing towards carbon neutrality.

Keywords: multi-carbon source co-fermentation; high-value chemical; synthetic biology; carbon neutrality

提升生物制造过程的碳原子经济性，是绿色生物制造领域亟待破解的核心科学问题之一，也是支撑国家“碳中和”目标落地的关键技术途径[1-5]。现有生物制造体系仍高度依赖葡萄糖和木糖等有机底物的异养代谢模式，工艺路径成熟但碳利用效率存在固有瓶颈。在天然中心碳代谢网络中，糖酵解途径与三羧酸循环是微生物介导底物分解代谢与前体合成的核心通路，

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其代谢过程高度依赖氧化脱羧反应，以此驱动碳骨架重排与能量供给[6]。该代谢模式存在显著的碳流失缺陷，约 30%–50% 的底物碳以 CO_2 形式不可逆释放，尤其在丙酮酸向乙酰辅酶 A 转化的关键节点，氧化脱羧反应直接造成碳原子经济性大幅下降[2,7]。上述由氧化脱羧不可逆反应与天然能量代谢的偏好特性带来的固有缺陷，不仅导致原料碳利用率偏低，更从本质上制约了生物基化学品与传统石化路线的成本竞争力。

当定向合成萜类[7-10]、长链脂肪醇[11-12]及中长链二羧酸[13-16]等高还原度与高能量密度的目标产物时，单一有机底物供给模式的固有缺陷会被显著放大，其体系局限性也进一步凸显。此类化合物的定向合成对碳骨架供给效率与还原力供应水平存在严格的化学计量匹配需求，而传统异养代谢途径存在路径相对固定与碳通量不足等缺点，难以同步满足细胞正常生长增殖与目标产物高效合成的双重物质及能量需求。高还原产物合成不仅需要持续且足量的碳骨架支撑分子链的延伸与组装，还依赖大量还原型辅酶推动关键还原反应进行，单一底物经戊糖磷酸途径等固有代谢通路产生的还原力通量有限，且受碳代谢物阻遏(carbon catabolite repression, CCR)效应调控，碳源会优先流向菌体基础代谢与生长繁殖，导致流向产物合成支路的碳流与还原力严重不足，进而引发胞内氧化还原稳态失衡以及中间代谢物积累等问题，最终造成原料转化率偏低、产物合成效率难以提升，无法满足实际发酵生产与工业化转化的应用要求。

为突破传统异养代谢存在的热力学瓶颈与动力学局限，研究人员聚焦低碳化合物与五碳及六碳糖的耦合代谢调控，并逐步发展形成一套重构微生物碳代谢网络[17-21]的新型技术体系，如图 1 所示。该策略通过理性整合甲酸、甲醇及合成气等 C1 碳源与乙酸等 C2 碳源，耦合有机底物代谢通路，构建物质互补、能量协同及氧化还原平衡的混合营养利用体系[22]。这类策略的核心逻辑是借助有机底物氧化分解释放的腺嘌呤核苷三磷酸(adenosine triphosphate, ATP)推动热力学层面难以自发进行的 C1/C2 固定过程，解除天然自养固碳过程中的能量约束；还可利用高还原态 C1 小分子碳源作为外源电子供体，调控胞内氧化还原稳态，完成碳流失节点的高效回补[23-25]。这类协同代谢机制打破了单一底物发酵的热力学局限，为工业尾气与生物质水解液在内的低值废弃碳源的高值化转化提供了全新的研究思路。合成生物学技术的快速迭代，加上理性设计理念的深度融入，推动了多碳源耦合体系逐步从概念验证阶段进入工程化应用阶段。与传统底盘菌株的自然筛选相比，现代工程化策略将重点放在底盘细胞的系统性代谢重编程上，涉及碳代谢重排途径[如非氧化糖酵解(non-oxidative glycolysis, NOG)途径]的设计与构建工作[26-28]、能量供给通路的精准匹配，以及碳代谢物阻遏等全局调控系统的优化[29-31]。本文全面梳理合成生物学赋能下多碳源耦合发酵领域的研究进展，重点分析有机底物与 C1/C2 小分子碳源协同代谢的分子机制和关键改造路径，说明其在高碳效细胞工厂构建及生物燃料和药物中间体在内的高值化学品合成中的应用潜力。同时，本文也梳理了该技术目前在实验室研究与工程化放大过程中面临的核心科学问题与技术挑战，并对其未来发展方向进行了展望，旨在为面向国家“碳中和”目标的新一代绿色生物制造技术发展提供有价值的理论参考与研究思路。

1 混合碳源发酵的代谢机制与分析

多碳源耦合发酵指微生物利用混合碳源，如五/六碳糖与非粮碳源(C1 与 C2 底物)的生物过程，通过代谢途径的协同调控可实现碳源高效利用，这类技术在合成生物学与代谢工程领域应用较广，能够提升高价值化学品的产出率

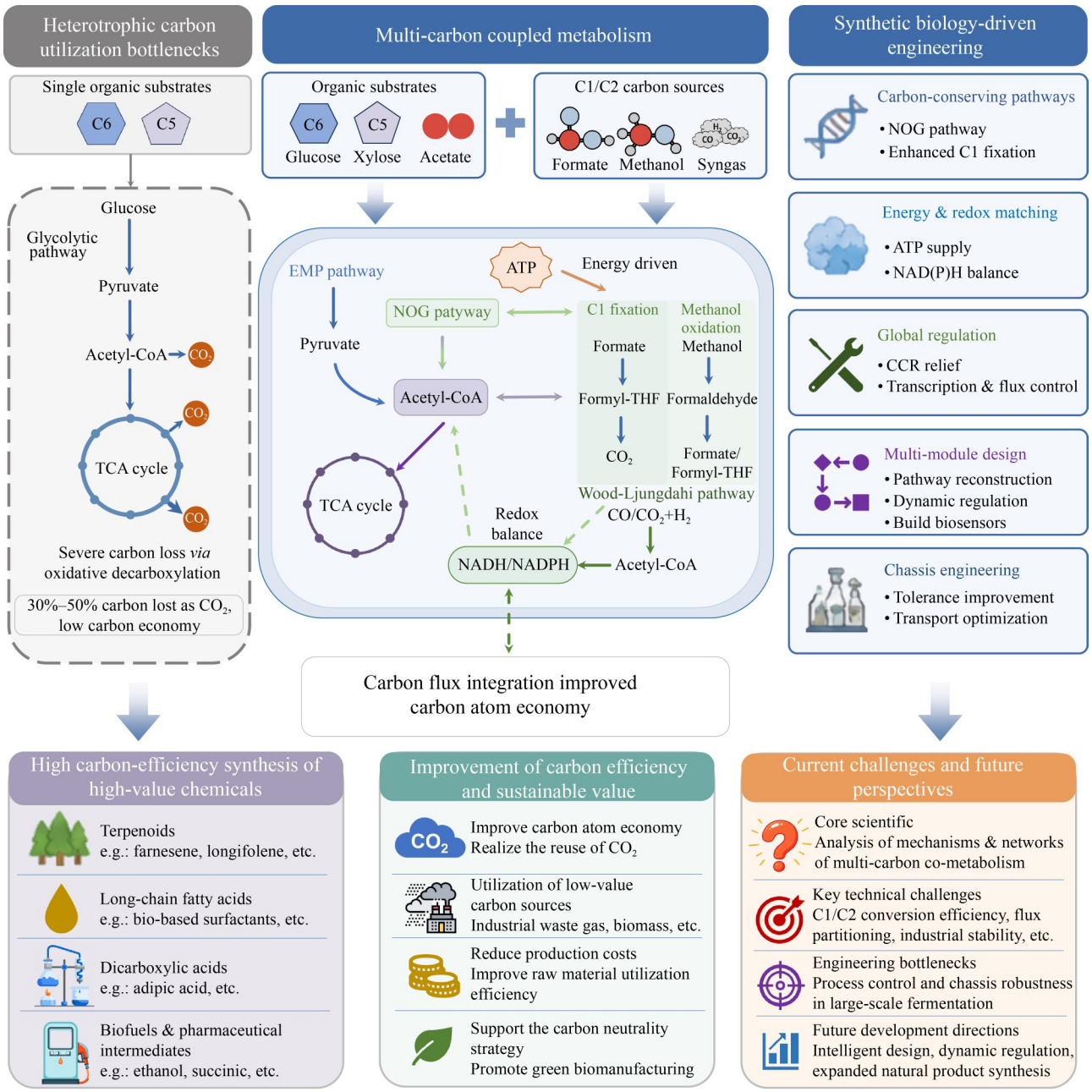

图1　多碳源耦合协同代谢策略示意图

Figure 1　Schematic of multi-carbon source coupled synergistic metabolism strategy.

与碳原子经济性[32]。与单一碳源工艺相比，多碳源耦合可优化能量分布，维持氧化还原稳态，依托相应调控机制降低碳损耗，可改善整体代谢效能[18,33]。后续将从能量耦合与热力学驱动机制、氧化还原平衡与电子载体适配、碳分解代谢物阻遏的分子机制及解除策略、碳代谢通量重定向与守恒机制 4 个维度展开论述。

1.1　能量耦合与热力学驱动机制

在微生物代谢多种碳源的过程中，能量耦合机制是碳源高效利用的核心前提，细胞具备

一套精细的能量调控系统，将分解代谢释放的能量载体(ATP)与合成代谢过程需要消耗的能量载体[合成蛋白质需要鸟苷-5′-三磷酸(guanosine-5′-triphosphate, GTP)，合成多糖需要尿苷三磷酸(uridine triphosphate, UTP)等]紧密衔接在一起[32]。混合碳源发酵环境中微生物会选择性利用不同碳源，匹配自身不同的能量需求，葡萄糖一般是优先被利用的碳源，经糖酵解途径可快速供给 ATP 以及还原力[34-35]；甘油可支撑细胞生长并提供特定氨基酸前体，使葡萄糖可更多用于目标产物合成，最终实现协同利用与高产[36]。这种碳源利用的功能分工可优化细胞的能量分配，在保障细胞正常活性的前提下尽可能提升目标产物的合成效率。

热力学驱动机制在多碳源代谢中同样发挥关键作用。生物体内反应的实际吉布斯自由能变(Δ*G*)决定了反应的方向和驱动力，其数值受细胞内代谢物浓度的动态调控[32]。通过调控关键代谢物的浓度，细胞可驱动标准条件下热力学不利的生化反应正向进行。研究表明，部分发酵反应本身热力学不利，但通过与互营微生物共培养可使原本难以自发进行的反应顺利发生，在共培养系统中一种微生物的代谢产物成为另一种微生物的底物，从而形成热力学上更有利的整体系统[33-34]。近年来，通过代谢网络重构和能量通量优化，研究者已能够显著提升目标产物的生产效率。例如，在大肠埃希氏菌(*Escherichia coli*)中通过引入异源转氢酶系统并优化电子传递链实现了还原型烟酰胺腺嘌呤二核苷酸磷酸[nicotinamide adenine dinucleotide phosphate, NAD(P)H]与 ATP 的协同再生，使 D-泛酸产量达到 86.03 g/L，产率达 0.80 g/(L·h)[35]。这种能量耦合与热力学优化对于提高混合碳源利用效率至关重要。

1.2 氧化还原平衡与电子载体适配

在微生物代谢过程中氧化还原平衡是维持细胞生命活动和产物合成的关键因素[36-37]。还原型烟酰胺腺嘌呤二核苷酸(nicotinamide adenine dinucleotide, NADH)和 NAD(P)H 是细胞内主要的还原力载体——NADH 主要参与分解代谢，而 NADPH 主要用于合成代谢和氧化应激过程[38]。多碳源耦合发酵可更精细地调控这些辅因子的生成和消耗，以实现氧化还原平衡的进一步优化[39]。

不同碳源在代谢过程中会产生不同比例的 NADH 与 NADPH。其中，木糖经磷酸戊糖途径代谢可产生更多 NADPH，这一特性对于木糖醇等需要大量还原力的生物合成途径尤为有利；而葡萄糖的代谢则主要通过糖酵解途径生成 NADH。因此，通过葡萄糖与木糖的混合利用微生物能够根据目标产物的特定还原力需求更为灵活地调控胞内 NADH 与 NADPH 的供应比例[40]。大量研究证实，胞内 NADPH 的高效再生是制约生物转化过程效率提升的主要限制因素之一[41-42]，传统的静态调控手段通常难以精准匹配动态的还原力需求，容易导致胞内 NADPH/$NADP^+$氧化还原对比例失衡，进而对细胞的正常生长与目标产物的生物合成产生不利影响[41]。Harth 等[42]以酿酒酵母(*Saccharomyces cerevisiae*)多碳源耦合发酵合成 L-半乳糖酸为模型，通过人工设计重构代谢途径定向调控胞内还原力供给，辅以酶工程改造改变关键酶辅酶偏好特性，精准匹配多碳源代谢与产物合成的还原力需求，实现胞内氧化还原平衡及目标产物高效合成。Wang 等[43-44]构建 CO_2-甲酸协同转化体系与酿酒酵母原料利用平台，补足还原力、优化碳同化效率，显著提升脂肪酸、乙醇等产物产量。另有研究耦合木糖代谢与 Rubisco 途径，结合木糖、麦芽糖共利用策略，改善辅酶失衡问题，有效提升木糖利用率与乙醇产率[45]。

此外，多碳源协同发酵还可通过将完整代谢途径模块化拆分至不同微生物菌株中，有效减轻单一底盘细胞的代谢负担，进而更为精准地调控胞内整体的氧化还原平衡状态[46]。例如，在大肠埃希氏菌共利用葡萄糖与甲酸的相关研究中通过对目标合成途径进行系统性优化，可

使丙酮酸产量得到显著提升[47]。这一研究结果充分表明，在混合碳源发酵体系中通过对代谢网络进行精细的动态调控，能够实现胞内碳通量与还原力供给的协同增效。

1.3 碳分解代谢物阻遏的分子机制与解除策略

碳分解代谢物阻遏是微生物在长期进化过程中形成的一种普遍调控机制。当环境中同时存在多种可利用碳源时，微生物通常会优先利用葡萄糖等有利生长的优势碳源。该机制主要通过转录水平的调控发挥作用，在葡萄糖存在的条件下会显著抑制木糖、乳糖、甘油等其他非优势碳源代谢相关基因的表达。这会导致微生物出现典型的二次生长现象，使得整体发酵周期延长，进而显著降低工业生产过程中的整体生产效率[48]。

根据调控元件的不同 CCR 的分子机制主要可分为两大类：一类依赖于磷酸烯醇丙酮酸-糖磷酸转移酶系统 (phosphoenolpyruvate-sugar phosphotransferase system, PTS)的磷酸化状态[49]，另一类则通过 CreA 等转录因子介导转录水平的调控。在大肠埃希氏菌中 PTS 系统是介导 CCR 效应的核心元件。其通过精准调控 EIIA 蛋白的磷酸化状态，进而影响腺苷酸环化酶的催化活性与胞内 cAMP 的合成水平，最终通过 cAMP 受体蛋白(cAMP receptor protein, CRP)介导乳糖操纵子等非优势碳源代谢基因的表达调控[49]。

为了克服 CCR 对多碳源耦合发酵的限制，研究人员开发了多种抑制解除策略。通过敲除 CCR 的关键调控基因(如 *pstG*[50]、*crr*[51]、*ccpA*[52]、*scrI*[53])开发了同时利用多种碳源的菌株。通过适应性进化和基因改造，研究人员可提高大肠埃希氏菌对木糖的利用效率，并改善葡萄糖/木糖的共利用，从而提高乙醇产量[54]。CCR 的调控模式并非绝对固化，而是具备显著的灵活性与环境适应性。这一结论可通过非葡萄糖碳源的激活效应得到直接佐证：在特定培养条件下向体系中添加少量葡萄糖并不会触发经典 CCR 效应中对非偏好碳源的代谢抑制，反而可有效激活微生物对半乳糖等非葡萄糖碳源的分解代谢通路，最终实现对 CCR 阻遏效应的显著缓解[55]。

1.4 碳通量重定向与守恒机制

碳通量重定向是代谢工程中的核心策略，旨在将碳流导向目标产物的合成途径，同时维持细胞的正常生长和活力[56]。在多碳源耦合发酵中这种重定向尤为关键，因为它需要平衡不同碳源的利用并协调多个代谢途径。

碳守恒调控机制是保障碳原子从底物到目标产物转化过程中实现最大利用效率的核心机制。理论上，同型乳酸发酵途径的碳收率可达到 100%，这是因为该途径能够将葡萄糖分子中的全部 6 个碳原子完整保留在乳酸产物中[57]。然而，在复杂发酵体系中碳损失是常见挑战，例如以 CO_2 形式逸出[58-59]。乙醇、甘油等高还原度非发酵性底物的引入，能够有效减少上述碳流失。这是由于这类碳源的氧化程度更低，在胞内代谢过程中产生的 CO_2 量显著低于葡萄糖等传统六碳糖底物。

在工业应用中有多种策略可实现碳通量的重定向与守恒。常见策略包括关键限速酶过表达与竞争性旁路敲除，通过增强限速酶活性或阻断竞争支路引导碳流流向目标产物合成途径。例如，在酿酒酵母中同步整合外源 β-葡萄糖苷酶和 β-木糖苷酶基因可实现纤维素来源糖类的共发酵，提升碳利用效率[60]。辅酶 NAD(P)H 在碳通量维持过程中作用突出，像 $NADP^+$依赖的甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase, Gapdh)可提升 3-羟基丙酸(3-hydroxypropionic acid, 3-HP)的产量，这是由于甘油作为底物可推动 NADPH 的再生[61]。通过构建合成微生物群落打造细胞工厂可将复杂代谢途径拆分至不同微生物中，每种微生物承担一个特定步骤，可降低单一菌株的代谢负担，提升整体碳利用效率[62]，譬如可构建利用

甲烷与木质纤维素糖合成四氢嘧啶的甲基营养菌平台。甲烷代谢可富余供给 NADPH，却存在碳骨架不足；木质纤维素糖类则能补充碳骨架并产生 NADH，二者还原力与碳代谢天然互补。通过代谢途径人工重构实现多碳源耦合发酵，精准匹配胞内还原力分配，大幅提升碳源利用率与目标产物合成效率，为甲烷和生物质糖联产胞外酶等高附加值产物提供了理论支持[63]。使用甘油这类非发酵性底物可减少传统发酵流程中 CO_2 的生成，提升碳原子经济性[64]，例如在双室生物反应器系统中将乙醇发酵产生的 CO_2 用于琥珀酸合成，可展现出碳循环利用的潜力[65]。

综上所述，混合碳源发酵通过能量与热力学调控、氧化还原稳态适配、解除碳代谢阻遏、碳通量优化多重机制协同作用，有效弥补单一碳源发酵缺陷，显著提升碳源利用效率与产物合成效能，为微生物发酵工业高效生产高价值产物提供了完善的理论支撑与技术思路(表 1)。

2 混合碳源发酵生产高值化学品的优化策略与应用进展

在“双碳”目标持续推进与石化资源替代需求日益迫切的背景下，微生物发酵驱动的生物制造已成为绿色合成高附加值化学品的核心技术路径，是推动化工产业低碳转型的重要方向。碳源是微生物菌体生长与目标产物合成的核心物质基础，其利用效率与原料成本直接决定发酵过程的经济性与工业化可行性。当前单一碳源发酵体系普遍存在技术瓶颈：粮食基葡萄糖面临原料成本高以及“与人争粮”的产业约束；木质纤维素来源的木糖等五碳糖易受碳代谢阻遏效应影响，天然利用效率不足；甲酸、甲醇、合成气以及乙酸等一碳和二碳底物虽来源广泛，低碳属性突出，却存在微生物代谢适配性差、产物合成得率低等问题。混合碳源发酵可通过多底物协同代谢实现不同碳源的优势互补(图 2)，可有效突破单一碳源的代谢局限，在降本增效和实现工业副产碳资源高值化利用等方面优势显著，已成为生物制造领域的研究热点。

2.1 有机底物与一碳化合物的氧化还原协同体系

甲酸作为典型的一碳(C1)化合物，在微生物混合营养代谢中可发挥多重核心生理功能：可经甲酸脱氢酶(formate dehydrogenase, Fdh)催化氧化生成 NAD(P)H，为胞内代谢提供电子与能量；可通过叶酸介导的 C1 代谢途径进入碳固定网络，为生物大分子合成直接提供碳骨架；还可在厌氧/微氧条件下缓解胞内氧化还原失衡，减少副产物积累，降低发酵过程的氧依赖。在甲酸与葡萄糖共利用的混合营养体系中葡萄糖主要承担碳骨架供给与基础能量供应的功能，甲酸则作为辅助碳源与电子供体实现碳代谢与能量产生的解偶联，进而显著提升碳利用效率，从而减少 CO_2 释放，强化目标产物的合成效率。目前，基于不同微生物底盘的甲酸-糖耦合代谢体系已得到广泛开发，为低碳生物制造提供了重要的技术支撑。

2.1.1 大肠埃希氏菌甲酸-糖耦合体系的代谢工程改造与产物合成

大肠埃希氏菌作为代谢工程改造的经典模式原核底盘，是甲酸-糖耦合发酵体系构建中应用最广泛的宿主。针对葡萄糖与甲酸协同利用效率不足的问题，Hu 等[47]通过代谢工程改造实现丙酮酸摩尔得率达 1.88 mol/mol 葡萄糖(糖酵解理论得率的 94%)；整合亚磷酸盐脱氢酶介导的 NADH 再生系统后，L-苹果酸摩尔得率提升至 1.65 mol/mol 葡萄糖，为高值有机酸合成提供了参考。针对高还原力需求产物的胞内还原力供给瓶颈，Bertrand 等[66]基于甲酸与氢气的电子解偶联策略阻断丙酮酸脱羧分支途径，利用氢气(86.60% 电子供给)与甲酸盐(98.40% 电子供给)实现还原力高效补偿，驱动碳通量重定向至乙醛酸旁路，使甲羟戊酸产量提升 57.60%，甲

表1 混合碳源发酵核心代谢机制与分析汇总表

Table 1 Summary table of core metabolic mechanisms and analysis of mixed carbon source fermentation

Core mechanism	Key scientific principles	Typical carbon source combinations and application cases	Industrial value and advantages
Energy coupling & thermodynamic driving	(1) Cells couple catabolic ATP with anabolic energy carriers (GTP/UTP) *via* precise regulation (2) Carbon sources divide functions to meet diverse energy demands (3) Key metabolite modulation drives thermodynamically unfavorable reactions (4) Co-culture syntrophy improves overall thermodynamic favorability	(1) Heterologous transhydrogenase system was introduced into *E. coli* with optimized electron transport chain to realize co-regeneration of NAD(P)H and ATP The titer of D-pantothenic acid reached 86.03 g/L with a productivity of 0.80 g/(L·h)[35] (2) Glucose and glycerol: glucose provides rapid energy supply, while glycerol sustains cell growth and generates amino acid precursors[36]	(1) Optimizes energy allocation, boosts product synthesis while sustaining cell viability (2) Breaks single-carbon-source energy bottleneck
Redox balance & electron carrier adaptation	(1) NADH dominates catabolism; NADPH functions in anabolism and oxidative stress (2) Different carbon sources yield distinct NADH/NADPH ratios (3) Multi-carbon coupling flexibly tunes intracellular reducing power ratio (4) Modular pathways reduce strain burden and enable precise redox control	(1) CO_2-formate co-conversion: 11.24% C1 assimilation, 0.48 g/(L·h) formate consumption, 10.10 g/L FFAs[43] (2) *S. cerevisiae* glucose-formate co-utilization: ethanol, FFAs, and longifolene yields up by 184%, 490%, and 100%[44] (3) mXR-XDH xylose and PRK-Rubisco CO_2 fixation modules: 0.47 g/g ethanol yield[45]	(1) Resolves reducing power imbalance from static regulation (2) Precisely matches product-specific reducing power demand (3) Synergizes carbon flux and reducing power supply
Molecular mechanism & relief strategies of carbon catabolite repression	(1) CCR drives preferential use of preferred carbons (e.g., glucose), represses non-preferred carbon metabolic genes, and causes diauxic growth (2) Core mechanisms: PTS phosphorylation regulation, transcriptional control by factors like *CreA* (3) Low glucose can activate rather than inhibit non-glucose carbon metabolism under specific conditions	(1) Knockout of key regulatory genes: *pstG*[50], *crr*[51], *ccpA*[52], *scrI*[53] (2) Adaptive evolution and engineering improve glucose/xylose co-utilization in *E. coli* and elevates ethanol yield[54] (3) Low-dose glucose activates galactose catabolic pathway[55]	(1) Eliminates diauxic growth and shortens fermentation cycle (2) Enables simultaneous efficient utilization of multiple carbon sources (3) Significantly improves overall industrial process efficiency
Carbon flux redirection & conservation mechanism	(1) Redirects carbon flux to target pathways while sustaining cell growth (2) Carbon conservation maximizes substrate-to-product carbon conversion efficiency (3) Low-oxidation carbons cut CO_2-related carbon loss (4) Synthetic consortia split pathways to lower single-strain burden	(1) Heterologous β-glucosidase and β-xylosidase genes were integrated into *S. cerevisiae* to achieve co-fermentation of cellulosic sugars[60] (2) Methylotrophs coupled with lignocellulosic sugars: methane provides NADPH while sugars supply carbon skeletons for ectoine biosynthesis[63] (3) Dual-chamber bioreactor: CO_2 generated from ethanol fermentation is recycled for succinic acid synthesis[65]	(1) Improves carbon economy and reduces carbon loss (2) Achieves natural complementation of reducing power and carbon metabolism (3) Supports high-value utilization of low-cost C1/C2 carbons (methane, CO_2)

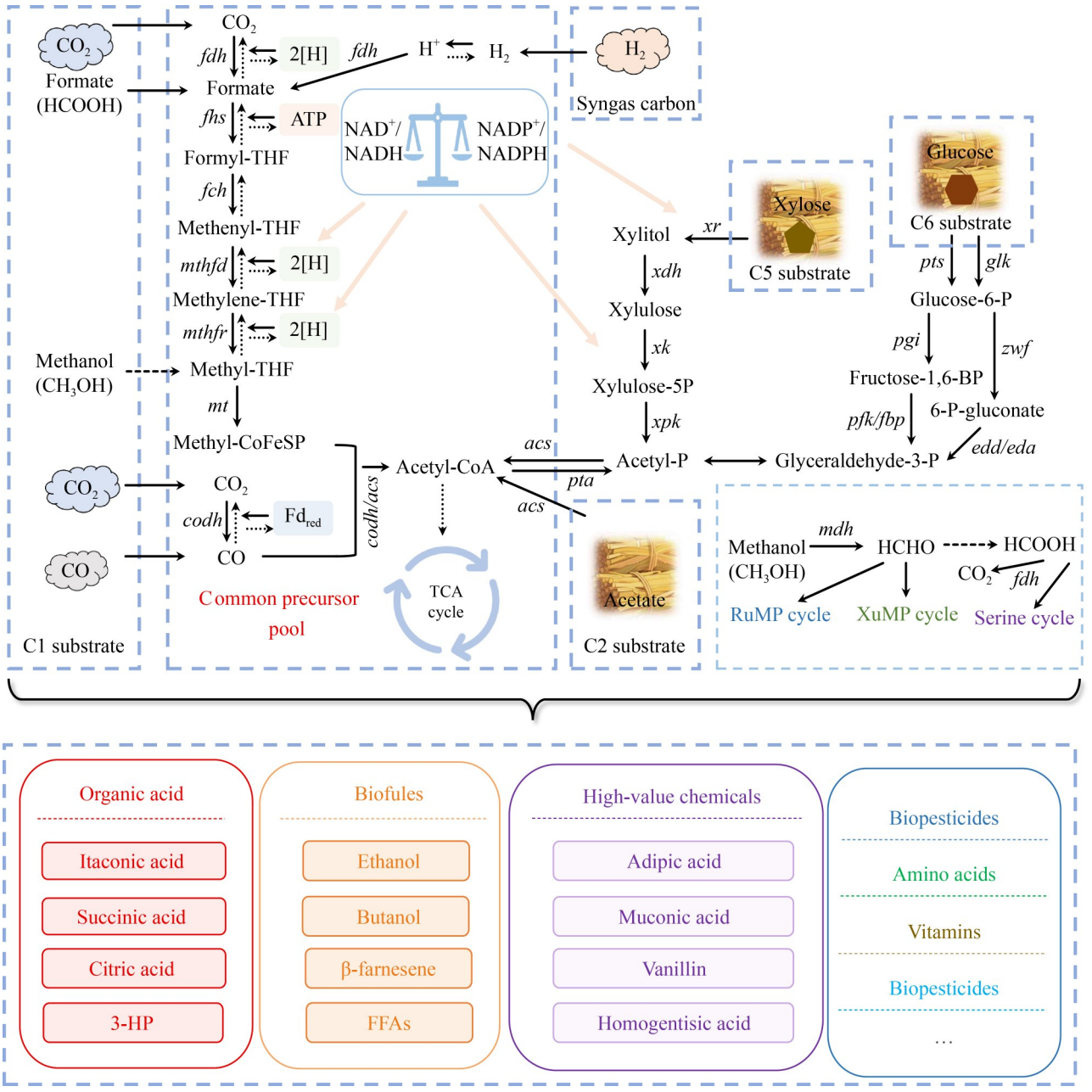

图2　混合碳源发酵生产高值化学品示意图

Figure 2　Mixed carbon source fermentation for high-value chemicals synthesis. Fdh: Formate dehydrogenase; Fhs: Formyltetrahydrofolate synthetase; Fch: Methenyltetrahydrofolate cyclohydrolase; Mthfd: Methylenetetrahydrofolate dehydrogenase; Mthfr: Methylenetetrahydrofolate reductase; Mt: Methyltransferase; Codh/Acs: Carbon monoxide dehydrogenase/acetyl-CoA synthase; Pta: Phosphotransacetylase; Pdc: Pyruvate dehydrogenase; Ack: Acetate kinase; Xr: Xylose reductase; Xdh: Xylitol dehydrogenase; Xk: Xylulokinase; Xpk: Phosphoketolase; Pts: Phosphotransferase system; Glk: Glucokinase; Pgi: Glucose-6-phosphate isomerase; Pfk/Fbp: Phosphofructokinase/fructose-1,6-bisphosphatase; Zwf: Glucose-6-phosphate dehydrogenase; Edd/Eda: 6-phosphogluconate dehydratase/2-keto-3-deoxy-6-phosphogluconate aldolase; Mdh: Methanol dehydrogenase.

酸电子利用率接近 99%，证实该体系适用于萜类与药物中间体合成。另有研究通过表达甲酸同化关键酶并筛选获得 K4M 突变株，甲羟戊酸(mevalonate, MVA)合成水平达 3.80 g/L，证实葡萄糖在菌体生长阶段对甲酸利用的辅助作用[67]。

2.1.2 真核微生物甲酸-糖耦合体系的代谢工程改造与产物合成

真核微生物凭借独特代谢特征在甲酸-糖耦合体系中展现出良好应用价值。解脂耶氏酵母(*Yarrowia lipolytica*)兼具优异耐酸能力与脂质合成优势，已成为核心真核底盘。Tao 等[68]将碳酸酐酶介导的 CO_2 再循环通路与甲酸代谢通路结合，构建 FDH-CA 工程菌株，实现琥珀酸产量 97.54 g/L、得率 0.64 g/g 底物，为闭环碳循环搭建提供了新方向；Van 等[69]研发绿色电力驱动的闭合碳循环技术，电催化 CO_2 还原为甲酸后与糖共饲喂酵母，在甲酸/糖物质的量比 5:1 条件下，300 L 中试产物得率比单一糖源体系高 20%，为近零碳排放生物制造提供了可行路径。Liu 等[70]利用甲酸、CO_2 基因工程改造大肠埃希氏菌，整合多功能代谢模块并建立两段式发酵；以甲酸与 CO_2 为主碳源、微量葡萄糖为辅，琥珀酸产量达 2.46 g/L，碳得率 0.43 mol/mol；木糖替代葡萄糖后，摇瓶产量 2.62 g/L、发酵罐产量 7.02 g/L，碳得率与 CO_2 固定量同步提升。Wang 等[43]搭建 CO_2 和甲酸协同转化通路，解决碳原子经济性不佳与木糖代谢还原力失衡问题，实现 C1 同化效率 11.24%、甲酸消耗速率 0.48 g/(L·h)，脂肪酸效价达 10.10 g/L，较对照分别提高 21.80 倍和 33.07 倍。后续研究构建了酿酒酵母 2G-3G 原料高效利用平台，利用甲酸补充还原力和胞内 CO_2 池实现 9.20% 的一碳同化效率，使乙醇、游离脂肪酸和长叶烯产率分别提高 18.40%、49.0% 和近 100%[44]。针对木质纤维素生物乙醇生产的木糖转化效率低和辅酶失衡难点，整合突变型木糖还原酶-木糖醇脱氢酶(mutated xylose reductase-xylitol dehydrogenase, mXR-XDH)代谢模块与磷酸核酮糖激酶-核酮糖二磷酸羧化酶耦合固碳途径(phosphoribulokinase-ribulose-1,5-bisphosphate carboxylase, PRK-Rubisco)，采用麦芽糖/木糖共利用与原位 CO_2 固定偶联策略，使木糖消耗率达 1.10 g/(L·h)，乙醇产率 0.47 g/g，较对照分别提高 104% 和 15%[45]。

2.1.3 非模式微生物甲酸-糖耦合体系的应用拓展

多种非模式微生物在甲酸-糖耦合体系中展现出良好场景适配性。Litsanov 等[71]通过敲除副产物基因、共表达丙酮酸羧化酶与甲酸脱氢酶，使谷氨酸棒状杆菌(*Corynebacterium glutamicum*)的琥珀酸浓度达 1 134 mmol/L，摩尔得率 1.67 mol/mol，副产物乙酸得率仅 0.10 mol/mol，该体系可显著降低发酵供氧需求，适用于厌氧/微氧生物合成。产油真菌 *Umbelopsis isabelline* 在甲酸/葡萄糖质量比 3.90:1 条件下生物量与脂质合成量分别提升 20% 与 70%；^{13}C 标记证实甲酸可同时提供还原力、能量并参与碳骨架合成，还能调控脂肪酸组分分布[72]。恶臭假单胞菌(*Pseudomonas putida*) KT2440 以甲酸为能量补充碳源时，生物量提升 25%，碳利用效率提高 19%，适用于极端环境下的生物合成[73]。

2.1.4 原核宿主甲醇-糖耦合代谢的构建与产物合成

除甲酸外，甲醇作为另一类优质非粮 C1 原料，其与糖的耦合利用策略也得到广泛研究。Witthoff 等[74]在大肠埃希氏菌中异源表达甲醇脱氢酶(methanol dehydrogenase, Mdh)与 RuMP 途径关键酶，阻断甲醛氧化支路，使甲醇同化率提升 8%–25%；葡萄糖/甲醇共底物条件下，甲醇消耗速率达 1.70 mmol/h，胞内 3%–10% 的碳来自甲醇。另有研究构建修饰的丝氨酸循环，实现甲醇/木糖强制共利用，乙醇产量达 1.89 g/L[75]。黏质沙雷氏菌(*Serratia marcescens*)天然具备萜类高耐受性，Wang 等[76]通过代谢工程优化，以甲醇/葡萄糖为共底物实现香叶醇产量 574.12 mg/L，碳足迹降低 40%；进一步整合双没

药醇合成酶后，α-红没药醇产量达 1 256.41 mg/L，为目前甲醇基萜类摇瓶发酵最高水平。Wu 等[77]构建体外木糖/甲醇耦合合成 L-乳酸途径，实现 100% 理论碳得率，经关键酶定向进化后碳转化效率达 88.75%，L-乳酸产量 6 g/L。随后在大肠埃希氏菌中构建新型甲醇同化途径，实现 2,4-二羟基丁酸(2,4-dihydroxybutanoic acid, 2,4-DHB)产量 14.60 g/L[78]。Sun 等[79]整合原核 Mdh 与真核二羟丙酮合酶(dihydroxyacetone Synthase, Das)构建杂合途径，实现甲醇向中心代谢物转化并伴随 NADH 再生，使(*R*)-1,3-丁二醇摇瓶产量达 5.79 g/L，批式发酵产量 13.71 g/L，碳摩尔得率 0.35 mol/mol；^{13}C 标记证实甲醇可同时作为碳源与还原力供体。

2.1.5 毕赤酵母甲醇-糖共代谢的机制解析与优化

在真核底盘中毕赤酵母(*Pichia pastoris*)是甲醇代谢的模式菌株。Jordà 等[80]采用代谢组与非稳态 ^{13}C 通量分析，解析了葡萄糖/甲醇(4:1)的共代谢机制，结果显示混合碳源条件下细胞密度提升 20%，磷酸戊糖途径(pentose phosphate pathway, PPP)氧化支路通量占 Glc6P 通量的 55%，甲醇同化率达 46%，系统能量效率显著提升。Li 等[81]基于转录组与代谢组分析揭示了毕赤酵母中甲醇与木糖代谢的正相关关系，通过磷酸盐供给优化实现甲醇利用率提升 34%，木糖利用率提升 357.50%，生物质干重达 7.50 g/L。

综上所述，甲酸、甲醇等 C1 底物与糖基底物的耦合利用(表 2)可通过协同供给还原力、补充碳骨架和调控胞内氧化还原平衡，有效突破单一碳源发酵的技术瓶颈，实现高值化学品的高效绿色合成。未来，通过关键酶的定向进化、代谢途径的动态精准调控以及发酵工艺的放大优化有望进一步提升 C1-糖耦合体系的代谢效率与经济性，为绿色生物制造的工业化落地提供有力支撑。

2.2 有机底物与二碳化合物的物质-能量互补利用体系

将有机底物与以乙酸为典型代表的二碳化合物结合形成的能质互补体系是合成生物学与代谢工程领域优化微生物细胞工厂性能的核心策略之一，这套体系的核心特点是能让不同底物的功能实现精准划分：葡萄糖和木糖等有机底物的主要功能是为微生物代谢提供碳骨架与基础能量，乙酸则充当辅助碳源与还原力供体，通过补充 NADH/NADPH 调控胞内的氧化还原平衡，完成碳流的重新分配，从而使得碳损失控制在较低水平，并且提高菌株的鲁棒性，最终优化目标产物的产量、得率与生产效率[82]。上述策略已在酵母与细菌等多种微生物底盘中得到普遍应用，为高值化学品的绿色生物制造提供了技术支持。

2.2.1 大肠埃希氏菌乙酸-糖能质互补体系的构建与高值化学品合成

大肠埃希氏菌作为经典原核模式的底盘，其能质互补体系的应用核心聚焦于碳流重定向与模块化途径构建，已在多类高值化学品合成中取得突破性进展。Yu 等[83]构建工程菌株 NZ-Gly303，采用模块化组装碳保守代谢途径实现乙酸与葡萄糖的协同高效转化，分批补料发酵中乙醇酸产量达 73.30 g/L，产率 1.04 g/(L·h)，总碳摩尔产率达理论值的 80%，为乙醇酸绿色合成提供了高效方案。Da 等[84]在丙酮酸合成研究中通过失活 *poxB*、*pflB*、*aceEF* 基因阻断竞争性途径，删除 *ldhA* 和 *mgsA* 消除乳酸副产物，工程菌株可从 10.27 g/L 葡萄糖与 2 g/L 乙酸中合成 9.61 g/L 丙酮酸。柠檬酸合成方面，通过敲除磷酸转移酶系统、优化乙酸同化途径及底物比例实现葡萄糖与乙酸的独立碳流定向合成，摇瓶产量达 5 g/L，摩尔产率 0.87 mol/mol[85]。另有研究构建氧化还原耦合途径并过表达乙酰辅酶 A 合成酶，实现葡萄糖与乙酸的化学计量共利用，丁酰丁酸酯产量达 29.02 g/L，碳产率

表2 有机底物与一碳化合物耦合生产高值化学品汇总表

Table 2 Summary of high-value chemical production *via* coupling organic substrates with one-carbon compounds

Strain	Substrate combination	Target product	Titer/productivity	References
Saccharomyces cerevisiae	Glucose/formate/CO_2	Fatty acids	10.10 g/L	[43]
Saccharomyces cerevisiae	Xylose/formate/CO_2	Ethanol, free fatty acids, and longifolene	18.40%, 49%, and nearly 100% increase	[44]
Saccharomyces cerevisiae	Maltose/glucose/CO_2	Ethanol	Productivity of 0.47 g/g	[45]
Escherichia coli	Glucose/formate	Pyruvate	1.88 mol/mol	[47]
Escherichia coli	Glucose/formate	L-malic acid	1.65 mol/mol	[47]
Escherichia coli	Glucose/formate/H_2	MVA	57.60% increase	[66]
Escherichia coli	Glucose/formate	MVA	3.80 g/L	[67]
Yarrowia lipolytica	Glucose/formate/CO_2	Succinic acid	97.54 g/L; 0.64 g/g	[68]
Yarrowia lipolytica	Glucose/formate/CO_2	Succinic acid	20% increase	[69]
Escherichia coli	Glucose/formate/CO_2	Succinic acid	2.46 g/L; 0.43 mol/mol	[70]
Corynebacterium glutamicum	Glucose/formate	Succinic acid	1 134 mmol/L; 1.67 mol/mol	[71]
Umbelopsis isabellina	Glucose:formate=1:3.90	Lipids	70% higher lipid titer	[72]
Pseudomonas putida KT2440	Glucose/formate	Biomass/polymer precursors	25% higher biomass	[73]
Escherichia coli	Glucose/methanol	Biomass	Methanol consumption: 1.70 mmol/h	[74]
Escherichia coli	Xylose/methanol	Ethanol	1.89 g/L	[75]
Serratia marcescens HBQA7	Glucose/methanol	Geraniol	574.12 mg/L	[76]
Serratia marcescens HBQA7	Glucose/methanol	α-bisabolol	1 256.41 mg/L	[76]
In vitro enzyme biosystem	Xylose/methanol	L-lactic acid	6 g/L; carbon efficiency of 88.75%	[77]
Escherichia coli	Glucose/methanol	2,4-DHB	14.60 g/L	[78]
Escherichia coli	Xylose/methanol	(*R*)-1,3-butanediol	13.71 g/L	[79]
Pichia pastoris	Glucose:methanol=4:1	Biomass	20% increase in cell density	[80]
Pichia pastoris	Xylose/methanol	Biomass	7.50 g/L	[81]

43.30%，为目前该产物生物合成的最高纪录[86]。谷氨酸棒杆菌通过丙二酰辅酶 A 途径改造从葡萄糖和乙酸中合成 3-羟基丙酸，产量较单一碳源提升 12.57 倍[87]。综上所述，乙酸作为来源广泛的廉价工业副产物，可通过平衡胞内辅因子含量、减少碳流失等机制显著提升大肠埃希氏菌的产物合成效率。

2.2.2 酿酒酵母乙酸-糖能质互补体系的还原力调控与代谢优化

酿酒酵母作为遗传背景清晰、工业应用最成熟的真核模式底盘，其能质互补体系的构建核心集中于胞内还原力平衡调控与乙酸代谢途

径的系统优化。Khattab 等[82]通过多基因组合改造实现多底物协同高效发酵：敲除乙醛脱氢酶基因 *ald6* 减少乙酸无效生成，异源引入树干毕赤酵母木糖同化途径并共表达木糖激酶降低副产物积累，导入肠炎沙门氏菌乙酰化乙醛脱氢酶 (*Salmonella enterica* acetylating acetaldehyde dehydrogenase, SeEutE)促进乙酸代谢与还原力耦合生成，同时敲除线粒体 NADH 脱氢酶基因阻断还原力无效消耗，过表达单羧酸转运体及突变型乙酰辅酶 A 合成酶强化乙酸摄取与同化；最终在微需氧条件下实现甘油、木糖、乙酸与葡萄糖的共发酵，4 种底物总乙醇转化率超过理论最大值的 95%，证实了多底物协同与还原力精准调控的叠加效应；同时进一步优化该体系，通过敲除 *gpd1* 弱化甘油合成、敲除 *ald6* 阻断乙醛向乙酸转化、删除 *nde1* 和 *nde2* 减少还原力流失，同时异源表达 SeEutE 增强乙酸代谢。在含 100 g/L 葡萄糖与 4 g/L 乙酸的培养基中，工程菌株可消耗 1 g/L 乙酸；经规律成簇的间隔短回文重复序列系统及相关蛋白 9 (clustered regularly interspaced short palindromic repeats, CRISPR-associated 9, CRISPR-Cas9)精准整合改造后，低氧条件下发酵速率提升 150%−343%，乙醇产量接近理论值，且菌株展现出乙醇诱导型絮凝特性。工程化酿酒酵母可实现低聚木糖、木糖与乙酸的协同利用，乙醇产量较单一糖源提升 84%[88]；上述研究表明，调控乙酸利用途径与胞内还原力平衡可有效避免氧化应激与碳源的无效流失，是提升酿酒酵母发酵性能的关键。

2.2.3 非常规微生物底盘乙酸-糖能质互补体系的应用拓展

非常规酵母与细菌底盘的能质互补体系，进一步拓展了该策略在木质纤维素转化等高值场景的应用潜力。解脂耶氏酵母作为典型产油酵母，Lin 等[89]通过整合异源木糖利用双路径、β-胡萝卜素合成模块、内源乙酰辅酶 A 合成路线及非氧化糖酵解通路构建木糖与乙酸协同代谢工程菌株，在 25 g/L 木糖和 25 g/L 乙酸钠培养基中 β-胡萝卜素产量达 776.90 mg/L (70.40 mg/g 干细胞重)，较单一木糖底物提升 4.20 倍；另一研究中，工程菌株在葡萄糖/乙酸混合底物中聚羟基丁酸酯(polyhydroxybutyrate, PHB)积累量达细胞干重的 10.20%，分批补料发酵产量达 7.35 g/L[90]。

东方伊萨酵母(*Issatchenkia orientalis*)具有天然耐酸性优势，工程菌株 IoDY01H 通过整合木糖利用与 β-丙氨酸合成途径实现木糖与乙酸协同转化，在 pH 5.50 条件下以大麻秸秆水解物为原料合成 3-羟基丙酸，产量达 8.70 g/L[91]。蓝色嗜盐单胞菌(*Halomonas bluephagenesis*)作为极端环境微生物底盘，Zhang 等[92]通过优化甲羟戊酸合成酶基因整合表达、利用葡萄糖与乙酸能质互补协调胞内还原力与能量平衡，结合成簇规律间隔短回文重复序列干扰技术(clustered regularly interspaced short palindromic repeats interference, CRISPRi)筛选关键调控靶基因并引入非氧化糖酵解途径，最优工程菌株摇瓶中甲羟戊酸产量达 13.90 g/L，5 L 反应器开放式流加发酵中进一步提升至 121 g/L，创下该产物生物合成的最高纪录。

2.2.4 微藻与特殊功能细菌能质互补体系的普适性研究

微藻与特殊功能细菌的能质互补体系验证了该策略的普适性。雨生红球藻(*Haematococcus pluvialis*)在葡萄糖与乙酸钠混合营养条件下生物量较单一碳源分别增加 77.10% 和 40.40%[93]；新绿藻(*Neochloris oleoabundans*)在 7.50 g/L 葡萄糖/乙酸钠和藻类最小盐培养基(bold basal medium, BBM)中生物量达 1.75 g/L，其中蛋白质含量占 24%−31%，脂质含量 34.40%，核磁共振分析显示游离脂肪酸占比 11%[94]。红冬孢酵母(*Rhodosporidium toruloides*)在葡萄糖与乙酸或木糖与乙酸共发酵中展现同步底物消耗特性，脂质含量达细胞干重的 56%，脂肪酸组成与可可脂相似[95]。凝结芽孢杆菌(*Bacillus coagulans*)发酵中补充 10 g/L 乙酸可显著上调三羧酸循环

等途径基因表达，生物量与乳酸产量分别提升17.60%和15.30%[96]。

2.2.5 乙酸-糖能质互补体系在木质纤维素全利用中的应用进展

乙酸-糖能质互补体系已成功拓展至木质纤维素全利用场景。在木质纤维素水解液的制备过程中半纤维素组分(以乙酰化木聚糖为主要成分)的脱乙酰化反应会释放大量乙酸；而纤维素在纤维素酶的酶解作用下则主要生成葡萄糖和木糖。由此形成的水解液体系天然存在有机底物与乙酸的混合碳源，为微生物的协同碳源利用提供了独特的物质基础。作为目前最具潜力替代粮食基原料的廉价可再生碳源，木质纤维素水解液的应用实践表明，有机底物与乙酸的能质互补利用体系已从实验室阶段的纯底物研究，成功推进至接近工业化应用的复杂天然底物体系，为绿色生物制造的规模化落地提供了切实可行的技术路径。如土曲霉(*Aspergillus terreus*)从纤维素水解液中合成衣康酸达39.60 g/L，质量得率为0.40 g/g，实现木质纤维素资源的全利用与高值化转化[97]。

综上所述，有机底物与二碳化合物的能质互补体系通过精准的途径工程、基因调控与底物比例优化有效解决了微生物发酵过程中的氧化还原失衡、碳源损失和毒性抑制等关键瓶颈(表3)，推动了从实验室研究到工业应用的技术转化。未来，结合多组学分析与人工智能(artificial intelligence, AI)辅助代谢路径设计等前沿技术将为绿色生物制造的可持续发展提供更广阔的应用前景。

2.3 气-液双相底物耦合发酵体系

2.3.1 合成气发酵技术概述与应用优势

合成气发酵是一类利用厌氧微生物将富含一氧化碳(CO)、二氧化碳(CO_2)和氢气(H_2)的气态底物转化为生物燃料与高值化学品的可持续生物加工技术。其原料可来源于生物质气化气、

表3 有机底物与二碳化合物耦合生产高值化学品汇总表

Table 3 Summary of high-value chemical production *via* coupling organic substrates with two-carbon compounds

Strain	Substrate combination	Target product	Titer/productivity	References
Saccharomyces cerevisiae	Glucose/acetate	Ethanol	95% of the theoretical value	[82]
Saccharomyces cerevisiae	Glucose/acetate	Ethanol	Titer close to theoretical maximum	[82]
Escherichia coli NZ-Gly303	Glucose/acetate	Glycolic acid	73.30 g/L; 1.04 g/(L·h)	[83]
Escherichia coli	Glucose/acetate	Pyruvate	9.61 g/L	[84]
Escherichia coli	Glucose/acetate	Pyruvate	5 g/L; 0.87 mol/mol	[85]
Escherichia coli	Glucose/acetate	Butyl butyrate	29.02 g/L; carbon yield of 43.30%	[86]
Corynebacterium glutamicum	Glucose/acetate	3-HP	12.57-fold	[87]
Saccharomyces cerevisiae	Xylose/acetate	Ethanol	84% higher product titer	[88]
Yarrowia lipolytica	Xylose/acetate	β-carotene	776.90 mg/L	[89]
Yarrowia lipolytica	Glucose/acetate	PHB	7.35 g/L	[90]
Issatchenkia orientalis	Xylose/acetate	3-HP	8.70 g/L (straw hydrolysate)	[91]
Halomonas bluephagenesis	Glucose/acetate	MVA	121 g/L (5 L bioreactor)	[92]
Haematococcus pluvialis	Glucose/acetate	Biomass	77.10% higher biomass	[93]
Neochloris oleoabundans	Glucose/xylose/acetate	Lipids	1.75 g/L; lipid content of 34.40%	[94]
Rhodotorula toruloides	Glucose (or xylose)/acetate	Lipids	56%; productivity of 0.19 g/(L·h)	[95]
Bacillus coagulans	Glucose/acetate	Lactic acid	15.30% increase in lactic acid titer	[96]
Aspergillus terreus	Cellulosic hydrolysate	Itaconic acid	39.60 g/L; 0.40 g/g	[97]

钢铁工业尾气和电石炉气等多种工业副产物，不仅可实现废弃碳资源的高值化利用，还能有效规避传统糖基发酵对粮食资源的依赖，是助力“双碳”目标实现的重要技术路径。

可利用合成气的微生物均具备一氧化碳脱氢酶(carbon monoxide dehydrogenase, Codh)与氢化酶(hydrogenase, H_2ase) 2 个核心功能酶。氢化酶催化 H_2 氧化生成还原当量提供电子；CODH 催化水煤气变换反应，将 CO 与 H_2O 转化为 CO_2 并释放还原当量，为碳固定与产物合成供能。目前可利用合成气的微生物主要包括产乙酸菌、光合细菌与好氧羧基营养菌，其中产乙酸菌应用最广泛，通过还原性乙酰辅酶 A (Wood-Ljungdahl, WL)途径固定碳，光合细菌与羧基营养菌则主要依赖还原型戊糖磷酸(Calvin-Benson-Bassham, CBB)循环[98]。

2.3.2 Wood-Ljungdahl 固碳途径代谢特征

WL 途径是已知 ATP 消耗最低的厌氧碳固定途径，也是合成气转化为乙酰辅酶 A 的核心路径，分为甲基与羰基 2 个分支。甲基分支中，CO_2 经甲酸脱氢酶还原为甲酸，与四氢叶酸结合生成甲酰-四氢叶酸(formyl-tetrahydrofolate, formyl-THF) (消耗 1 mol/L ATP)，再经连续还原反应生成甲基-四氢叶酸(5-methyltetrahydrofolate-tetrahydrofolate, 5-methyl-THF)，最终由钴胺素铁硫蛋白转移至一氧化碳脱氢酶-乙酰辅酶 A 合酶(carbon monoxide dehydrogenase-acetyl-coA synthase, CODH-ACS)复合体。羰基分支中，CO_2 经 CODH 催化还原为羰基，或直接由 CO 转化而来。最终在 CODH-ACS 复合体中羰基、甲基与辅酶 A 结合生成乙酰辅酶 A[99]。

2.3.3 基于乙酰辅酶 A 的产物定向合成与代谢改造

乙酰辅酶 A 是合成气发酵产物多样化的核心节点，可转化为多种高值化学品。食一氧化碳梭菌(*Clostridium carboxidivorans*)过表达 *adhE2* 单基因可使乙醇产量达 3 g/L (较野生型提高 50%)，共表达 *adhE2* 与 *fnr* 可使丁醇产量提升至 0.35 g/L[100]。调控培养条件后己醇产量可达 1.90 g/L，外源补充乙醇后进一步提升至 2.34 g/L。永达尔梭菌(*Clostridium ljungdahlii*)经多基因簇整合改造可直接以 CO_2/H_2 为唯一碳源合成己醇与丁醇[101]。对永达尔梭菌进行多基因簇整合改造，成功以 CO_2/H_2 为唯一碳源能源合成己醇与丁醇，2 L 发酵罐中己醇产量达 0.39 g/L[102]。同时将醛脱氢酶(aldehyde dehydrogenase, Aldh)同工酶精准失活改造，成功以合成气($CO/CO_2/H_2$)为唯一碳源能源合成乙醇，生物反应器中乙醇产量较野生型提升 2−5 倍，乙酸选择性下降 60%−80%[103]。Yang 等[104]采用三步式代谢工程改造永达尔梭菌，强化乙醇合成、激活乙酸再同化、阻断 2,3-丁二醇副产物。工程菌以合成气为唯一碳源，2 L 罐乙醇产量达 30.10 g/L。此外，丙酮丁醇梭菌通过内源途径可实现 3-羟基丁酸产量 9.25 g/L[105]，为乙酰辅酶 A 向高值化学品转化提供了新的可行路径。

2.3.4 合成气发酵传质瓶颈与增效调控策略

合成气发酵为典型的气-液两相反应体系，CO、H_2 等气态底物在水相中的溶解度极低，气液传质效率是限制底物利用率与发酵效率的核心瓶颈。目前研究者主要通过反应器结构优化、操作参数调控和培养基改良等策略提升传质效率与发酵性能。在反应器优化方面，搅拌釜反应器是目前应用最广泛的反应器体系，通过优化搅拌速率、气体流量与回流系统[106]，可有效提升气液接触效率，例如筛选获得的霍氏肠杆菌(*Enterobacter hormaechei*) RF2 菌株，在厌氧培养瓶中可合成 21.80 g/L 生物乙醇，在优化后的搅拌釜反应器中产量可达 25.30 g/L，这也是首次报道该菌株在合成气发酵中试规模应用的潜力[107]；双反应器策略将菌体生长与产物合成阶段分离，可使黏液真杆菌(*Eubacterium limosum*)的碳转化率提升至 9.30 mmol/h，乙酸浓度达 34.40 g/L[108]；此外，合成气发酵与 CO_2 电解技术结合形成串联工艺，通过气体扩散电极结构工程可制备组成可调的合成气，为中链

脂肪酸合成提供可控底物供给。

近年来，合成气发酵技术在代谢机制解析、工程菌株改造及发酵过程优化等关键维度取得显著进展，为工业尾气等废弃碳资源高值化转化与碳中和绿色生物制造体系构建提供了可行技术路径(表 4)。然而，该技术工业化仍面临气液传质效率低、产物合成速率与浓度不足、菌株对有毒底物耐受性差导致发酵稳定性弱等核心瓶颈。未来需通过多学科交叉构建高性能工程菌株，开发新型反应器与原位分离技术，并结合电催化-生物转化耦合的人工碳循环技术，推动其规模化工业应用。

2.4 非糖基短链碳源耦合利用体系

在合成生物学与代谢工程领域，甲醇、甲酸及乙酸等非糖基短链底物正逐步替代传统糖类碳源，成为可持续生物制造领域的研究热点。此类底物可来源于工业有机副产物以及 CO_2 电/化学还原产物等多种非粮渠道，不仅原料成本低廉，还可有效降低生物制造过程对粮食资源的依赖，契合“双碳”目标下绿色制造的发展需求。单一短链底物的微生物转化普遍存在瓶颈，如甲醇和甲酸等 C1 底物存在同化效率低、中间代谢产物毒性强、胞内还原力与能量代谢失衡的问题，乙酸等 C2 底物则易引发 pH 胁迫、菌体生长与产物合成耦合度低等局限。通过不同短链底物的耦合利用，可实现底物间的代谢互补，平衡胞内碳骨架供给、还原力与能量代谢，有效缓解单一底物的毒性效应与代谢限制，提升碳利用效率与目标产物合成水平。该策略的核心在于通过同化途径重构、关键酶表达优化与发酵环境调控实现多底物的协同同化，目前已在多种微生物底盘中得到验证与应用。

2.4.1 C1-C1 与 C1-C2 底物耦合的代谢构建与产物合成

针对 C1 底物协同利用，研究者已在毕赤酵母与酿酒酵母中异源构建甲醇/甲酸同化的甲醇-甲酸氧化耦合还原性甘氨酸(methanol-formate oxidation coupled reductive glycine, MFORG)途径，实现甲醇、甲酸盐与 CO_2 的协同转化。工程化毕赤酵母可合成 0.71 mg/L 5-氨基乙酰丙酸(5-aminolevulinic acid, 5-ALA)与 0.21 g/L 乳酸，酿酒酵母工程菌株的 ALA 与乳酸产量分别达 1.67 mg/L 与 0.07 g/L。^{13}C 示踪实验显示，产物中来自甲醇和 CO_2 的碳元素标记占比超过 91%，验证了该途径对 C1 底物的高效定向转化能力，同时增强了宿主对 C1 底物的耐受性，为酵母底盘的 C1 底物高效利用提供了新方向[109]。原核甲基营养菌作为底盘时，C1 与 C2 底物耦合也展现出显著优势。甲基营养菌扭托甲基杆菌(*Methylorubrum extorquens*) AM1 作为甲醇利用模式菌株，经改造合成紫罗兰素，仅以甲醇为唯一碳源时产量为 11.70 mg/L，经随机诱变和定

表4 合成气生产高值化学品汇总表

Table 4 Summary of high-value chemical production from syngas

Strain	Substrate combination	Target product	Titer/productivity	References
Clostridium carboxidivorans	Syngas	Ethanol	3 g/L	[100]
Clostridium carboxidivorans	Syngas	Butanol	0.35 g/L	[100]
Clostridium carboxidivorans	Syngas	Hexanol	1.90 g/L	[101]
Clostridium ljungdahlii	CO adjusted to 70%	Hexanol	0.39 g/L	[102]
Clostridium ljungdahlii	CO_2/H_2	Hexanol	2−5-fold	[103]
Clostridium ljungdahlii	Syngas	Ethanol	30.10 g/L (2 L fermenter)	[104]
Clostridium acetobutylicum ATCC 824	Syngas	3-HP	9.25 g/L	[105]
Enterobacter hormaechei RF2	Syngas	Ethanol	25.30 g/L (reactor)	[107]
Eubacterium limosum KIST612	Syngas	Acetic acid	34.40 g/L	[108]

点突变优化关键途径后产量翻倍，进一步耦合乙酸盐作为辅助碳源后，产量大幅提升至118 mg/L；该研究证实C1/C2底物耦合可突破单一甲醇底物造成的莽草酸途径代谢瓶颈，解决菌体生长滞后问题，为甲基营养菌底盘的高值化应用提供新方法[110]。在*E. limosum*中，Wood等[111]发现甲醇与甲酸的比例可定向调控产物的合成方向，分批发酵中低甲醇-甲酸比例的底物组合以丁酸盐合成为主，底物消耗速率达220 mmol/(g DCW·d)，提高甲醇比例后代谢通量转向丁醇合成，丁醇产量达2 mmol/L；底物消耗呈明显的两阶段特征，发酵初期以甲酸利用为主，后期需平衡甲醇与甲酸的消耗比例，提示丁醇合成来自溢出代谢，而甲醇在胞内NADH供给中具有独特作用，该研究解析了多底物耦合的动态代谢规律，为厌氧发酵过程的底物调控提供了理论支撑。

2.4.2 甲酸-乙酸耦合体系在生物聚合物与生物燃料合成中的应用

甲酸与乙酸耦合利用可充分结合C1底物的还原力供给优势与C2底物的碳骨架供给优势，在生物聚合物及生物燃料合成领域展现出巨大应用潜力。在生物聚合物合成方面，从土壤中分离的格萨假单胞菌(*Pseudomonas gessardii*)天然具备利用乙酸盐与甲酸盐的能力，以5 g/L乙酸盐为碳源时菌体生长状态最优。经表达C1同化关键酶后，菌株对甲酸盐的同化速率显著提升，补料分批发酵中合成的中长链聚羟基脂肪酸酯(medium chain length-poly-β-hydroxyalkanoate, mcl-PHA)产量达0.40 g/L，占细胞干重的30.43%，为工业废酸等廉价底物资源化合成生物可降解材料提供了可行技术路径，拓展了短链底物耦合体系的适用场景[112]。生物燃料合成研究中，酿酒酵母经代谢工程改造后以CO_2衍生物甲酸和乙酸充当底物，合成生物燃料前体游离脂肪酸(free fatty acids, FFAs)。Wang等[113]解析并优化了菌株利用甲酸、乙酸的途径与FFA合成策略，最终构建的工程菌株的FFA滴度提升8倍，最高为6.60 g/L，给废弃碳源资源化与绿色燃料生产提供了新路径。同时Bi等[114]又搭建了电催化-微生物转化耦合系统，借助固态电解质电催化体系将CO_2还原为乙酸与甲酸，再将二者作为碳源饲喂工程化解脂耶氏酵母，试验中乙酸为β-法尼烯合成提供碳骨架，甲酸为胞内代谢提供还原力，额外添加多聚磷酸盐构建ATP再生系统；最终，该体系实现了14.80 g/L β-法尼烯的高效合成，全流程光能到产物的能源转化效率达0.75%；该研究将电催化CO_2固定与微生物转化过程进行了高效耦合，为可再生电力驱动的绿色生物燃料合成提供了全新的发展模式。

2.4.3 短链底物耦合的核心代谢机制解析

为进一步提升短链底物的同化效率，研究者对底物耦合的核心代谢机制进行了深入挖掘，在大肠埃希氏菌中，Zelcbuch等[115]通过激活丙酮酸甲酸裂解酶(pyruvate formate lyase, Pfl)的逆反应实现了乙酸盐与甲酸盐的高效同化，在乙醛酸循环分流缺陷的菌株中，Pfl逆反应可驱动乙酸与甲酸合成丙酮酸，显著提升菌株的生长速率，^{13}C标记实验证实了菌株生长对Pfl逆反应途径的依赖性，该途径可有效提升胞内ATP的产出效率，为短链底物的同化提供了新的路径。

2.4.4 短链底物的细胞毒性与宿主耐受性研究

短链有机酸的细胞毒性是限制底物耦合体系效率的核心瓶颈之一，不同微生物底盘对乙酸和甲酸的耐受性存在显著差异，混合底物的协同毒性效应也受到广泛关注。产溶剂梭菌相关研究表明，丙酮丁醇梭菌(*Clostridium acetobutylicum*)对乙酸与甲酸的耐受性阈值较低：3.70−9.70 g/L的乙酸可小幅提升丙酮-丁醇-乙醇(acetone-butanol-ethanol, ABE)发酵产量，但浓度超过11.70 g/L时产物合成显著受抑；甲酸的细胞毒性更强，0.40 g/L的甲酸即可使ABE产量下降77%，1 g/L甲酸可使产量下降25%；当二

者共存时(8.70 g/L 乙酸和 0.40 g/L 甲酸) ABE 产量从 15.10 g/L 降至 8.60 g/L，呈显著的协同毒性效应。拜氏梭菌(*Clostridium beijerinckii*)在相同底物条件下可维持稳定的溶剂合成能力，证实其对短链有机酸具有更强的耐受性；该研究为含酸木质纤维素水解物等廉价底物的发酵利用提供了宿主筛选依据，也提示宿主的耐受性改造是短链底物耦合体系优化的重要方向[116]。

2.4.5 短链底物耦合体系的规模化发酵应用验证

短链底物耦合利用的相关策略已在规模化发酵中得到了综合应用验证，以除草剂前体 L-高丝氨酸的合成为例，Zhang 等[117]以大肠埃希氏菌为底盘，通过系统代谢工程改造实现了乙酸盐与甲酸盐的高效协同转化，首先通过敲除分支代谢途径、激活乙醛酸循环和强化 L-高丝氨酸合成核心途径，构建了初始合成菌株，再通过自适应进化提升菌株对短链有机酸的耐受性，最后通过弱化糖异生途径优化胞内碳通量分布，在 5 L 发酵罐的补料分批发酵中仅以乙酸盐与甲酸盐为碳源，实现了 15.96 g/L L-高丝氨酸的高效合成，该研究整合了途径重构、耐受性改造和通量优化等多种策略，验证了 CO_2 衍生短链底物规模化合成高值氨基酸的可行性，为短链底物耦合体系的工业应用提供了范例。

甲醇、甲酸、乙酸等非糖短链底物的耦合利用策略，通过代谢互补突破单一底物的毒性效应与代谢瓶颈，平衡胞内碳骨架、还原力与能量代谢，显著提升非粮碳源转化效率，在生物燃料、可降解材料及医药中间体等高值产物合成中展现出广阔前景(表 5)。目前该领域虽已取得诸多进展，但仍面临底盘耐受性与同化效率不足、多底物协同代谢机制未完全解析、规模化工艺稳定性与经济性待优化等挑战。未来需结合多学科技术手段构建高性能工程菌株，开发电催化-生物转化耦合工艺，推动其在绿色生物制造中的应用，助力“双碳”目标实现。

3 总结与展望

合成生物学驱动的多碳源耦合发酵技术在实验室层面已实现较高的碳转化效率，展现出替代传统发酵工艺、提升生物制造碳利用率的巨大潜力。然而，该技术从概念验证走向工业化放大与产业化应用，仍面临底盘适配性、代谢调控精准性、设计理性化程度及过程经济性等多重挑战，亟待从系统层面突破关键技术

表5 非糖基短链碳源耦合生产高值化学品汇总表

Table 5 Summary of high-value chemical production *via* coupling non-sugar short-chain carbon sources

Strain	Substrate combination	Target product	Titer/productivity	References
Pichia pastoris	Methanol/famate/CO_2	5-aminolevulinic acid	0.71 mg/L	[109]
Saccharomyces cerevisiae	Methanol/famate/CO_2	Lactic acid	0.21 g/L	[109]
Methylorubrum extorquens AM1	Methanol/acetate	Violacein	118 mg/L	[110]
Eubacterium limosum	Famate/methanol	Butanol	2 mmol/L	[111]
Pseudomonas gessardii	Famate/acetate	Medium-chain-length polyhydroxyalkanoates (mcl-PHA)	0.40 g/L	[112]
Saccharomyces cerevisiae	Famate/acetate	Fatty acids	6.60 g/L	[113]
Yarrowia lipolytica	Famate/acetate/electrocatalysis CO_2	β-farnesene	14.80 g/L	[114]
Escherichia coli	Famate/acetate	Biomass	–	[115]
Clostridium acetobutylicum	Famate/acetate	Acetone-butanol-ethanol (ABE)	8.60 g/L	[116]
Escherichia coli	Famate/acetate	L-homoserine	15.96 g/L	[117]

瓶颈。

底盘细胞在复杂工业环境下的鲁棒性不足，是制约耦合发酵工艺放大的核心问题[118]。工业生物炼制原料(如木质纤维素水解液与工业尾气等)组分波动显著[119]，且含有酚类、呋喃类与高浓度有机酸等代谢抑制物[120-122]，易造成胞内代谢紊乱、生长抑制乃至细胞死亡，难以维持人工构建的多碳源耦合代谢网络稳定运行。因此，亟须推进底盘细胞全局抗逆改造与环境适应性进化研究[123]。一方面，可借鉴全局代谢网络鲁棒性工程策略，对中心代谢、胁迫响应与能量供给系统进行模块化重构，提升菌株对复杂环境的耐受能力[124-125]；另一方面，结合适应性实验室进化与理性设计[126-128]，靶向改造膜转运系统、胁迫感应调控通路及解毒代谢模块，构建可耐受高浓度甲酸、乙酸及复合杂质的高性能底盘[129-130]，维持底盘菌株的代谢稳态。

多底物代谢流的动态失衡与中间代谢物累积是限制碳转化效率进一步提升的关键障碍[131]。不同碳源在跨膜转运速率、代谢途径动力学及辅因子需求上存在显著差异[18,132]，易导致甲醛、乙醛等毒性中间体积累[133]以及 NAD(P)H/ATP 平衡失调[133-134]，造成碳流分配效率下降。未来研究应聚焦智能化动态调控网络构建[135-136]，开发针对关键中间代谢物(如甲羟戊酸或丙酮酸等)、胞内辅因子及氧化还原状态的高灵敏度生物传感器[137-139]，耦合光遗传开关与代谢拨动开关等正交基因线路[140-141]，建立多碳源摄取与代谢流分配的实时反馈系统，实现多底物代谢的自适应协同与精准调控[142-144]。

数据驱动的理性设计体系尚未完善，仍是制约多碳源耦合体系高效构建的主要短板[145]。传统菌株改造依赖局部通路优化，难以实现全局代谢通量重排[146-147]，且设计-构建-测试-学习(design-build-test-learn, DBTL)循环周期长以及试错成本高[148-149]。随着生物信息学与人工智能技术的深度融合[150]，研究者基于基因组尺度代谢网络模型(genome-scale metabolic model, GSMM)结合机器学习算法可实现多碳源条件下全局代谢通量的精准预测与关键靶点挖掘[151-152]；通过深度学习辅助 Rubisco 和 Fdh 等关键限速酶的定向进化，可显著提升酶催化效率与底物适配性[153-155]，从而缩短菌株迭代周期，提升合成生物学设计的理性程度与高通量应用水平[156]。

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