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Review 综述

甲醇生物转化合成化学品的代谢工程策略与研究进展

孙晓梅，王昕，陈可泉*

南京工业大学 生物与制药工程学院，江苏 南京

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摘　要：实现碳达峰碳中和是我国的重要目标。以甲醇、二氧化碳、甲酸等碳一(C1)化合物为原料的第三代生物制造逐渐受到关注。甲醇具有储存运输方便、还原力高、可通过 CO_2 氢化大规模制备等优势，被认为是理想的生物制造原料。本文聚焦天然及非天然甲醇同化路径，系统比较不同路径的优势；进一步总结天然甲醇利用微生物的甲醇代谢路径及非天然甲醇利用微生物的路径设计策略，并探讨甲醇利用过程中面临的问题。在此基础上，总结利用甲醇合成各类高附加值化学品的研究进展，进一步归纳甲醇生物转化中的关键限速点及工程改造策略。本文将为甲醇驱动的绿色生物制造提供理论支撑。

关键词：甲醇；代谢路径；高附加值化学品

Metabolic engineering strategies and research advances in synthesis of chemicals through bioconversion of methanol

SUN Xiaomei, WANG Xin, CHEN Kequan*

College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing, Jiangsu, China

Abstract: Achieving carbon peaking and carbon neutrality is a major strategic priority for China. Third-generation biomanufacturing, which uses one-carbon (C1) compounds such as methanol, carbon dioxide, and formic acid as feedstocks for bioconversion, has attracted increasing research

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*Corresponding author. E-mail: kqchen@njtech.edu.cn
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interest. Among these C1 feedstocks, methanol is considered a promising substrate for biomanufacturing because of its ease of storage and transportation, high degree of reduction, and potential for large-scale production *via* CO_2 hydrogenation. This review systematically compares natural and synthetic methanol assimilation pathways and elucidates their advantages. It further summarizes methanol metabolism in natural methylotrophic microorganisms and pathway design strategies for constructing synthetic methylotrophs, while also discussing the major challenges associated with methanol utilization. On this basis, this paper reviews recent progress in the methanol-based biosynthesis of various high-value chemicals and discusses the bottlenecks and corresponding engineering strategies for methanol bioconversion. This review provides a theoretical foundation for methanol-driven green biomanufacturing.

Keywords: methanol; metabolic pathway; high-value chemical

在全球气候变化及化石资源枯竭的双重挑战下，构建以“碳中和”为核心的循环经济体系已成为全球共识[1-2]。以二氧化碳(CO_2)、甲醇、甲酸等碳一(C1)化合物为原料的第三代生物制造技术，因其能够利用非粮碳源、减少对化石资源的依赖、实现温室气体资源化利用等优点，受到广泛关注，在绿色制造与可持续发展领域展现出重要意义[1-2]。在众多 C1 原料中，甲醇具有来源丰富、储存运输便利、还原性强等优势，同时可通过 CO_2 加氢实现大规模制备，被视为推动生物制造向绿色低碳方向转型的理想原料[3]。

微生物细胞对甲醇的高效利用是甲醇生物转化产业化的核心[4]。围绕这一目标，近年来国内外学者开展了大量研究，主要分为 2 条技术路线：一类是开发和改造天然甲基营养菌，另一类是构建合成型甲基营养菌[4]。天然甲基营养菌，包括食甲基丁酸杆菌(*Butyribacterium methylotrophicum*)、法夫驹形氏酵母(*Komagataella phaffii*)、扭托甲基杆菌(*Methylorubrum extorquens*)、甲醇芽孢杆菌(*Bacillus methanolicus*)、多形汉逊酵母(*Ogataea polymorpha*)、博伊丁假丝酵母(*Candida boidinii*)等[4-8]，能够以甲醇为唯一碳源和能源直接生长，具有完整的甲醇氧化与同化代谢网络，在氨基酸(如丝氨酸、谷氨酸、赖氨酸)、有机酸、生物燃料及单细胞蛋白(single cell protein, SCP)等产物合成方面已展现出应用潜力[9]。然而，该类菌株普遍存在遗传操作体系不完善、代谢调控复杂及产物谱受限等问题[4]。与此同时，基于模式菌株构建合成型甲基营养菌被视为实现甲醇高效生物转化的另一有效途径[4]。研究者利用成熟的遗传操作工具和系统代谢工程策略，通过引入或重构甲醇同化途径，实现了对 C1 代谢的可控设计与优化，目前已成功在大肠埃希氏菌(*Escherichia coli*)[10]、酿酒酵母(*Saccharomyces cerevisiae*)[11]、谷氨酸棒状杆菌(*Corynebacterium glutamicum*)[12]等底盘细胞中构建出合成型甲基营养菌，为甲醇生物转化提供了新的平台。

近年来，随着合成生物学技术的快速发展，甲醇生物转化体系在代谢效率、产物种类及工艺水平方面均取得显著进展。以甲醇为原料的生物制造已逐步拓展至有机酸、生物燃料、氨基酸及医药中间体等多个高附加值化学品领域，显示出良好的应用前景[9]。然而，甲醇毒性强、代谢通量低以及还原力与能量分配失衡等问题仍是其规模化应用面临的关键瓶颈[4,13]。基于此，本文系统梳理了甲醇生物转化的研究进展，重点综述天然与人工甲醇同化途径的代谢特征与工程优化策略，比较分析不同微生物底盘在甲醇利用中的优势与局限，并总结甲醇在高附

加值化学品合成中的最新应用进展，最后对该领域未来的发展方向与关键挑战进行展望，以期为构建高效、可持续的 C1 生物制造体系提供参考。

1 天然甲醇同化途径

自然界中，已发现多种微生物具备甲醇等碳一化合物的利用能力，包括有氧代谢过程和厌氧代谢过程，其代谢路径呈明显的物种特异性(表 1)。

1.1 有氧甲醇同化途径

在有氧条件下，甲醇的代谢过程可分为甲醇氧化与碳同化 2 个阶段[22]。首先，甲醇在不同类型的脱氢酶或氧化酶的催化作用下生成甲醛，根据酶学特性，这些酶主要包括以下 3 类：(1)吡咯喹啉醌(pyrroloquinoline quinone, PQQ)依赖的甲醇脱氢酶，主要存在于革兰氏阴性甲基营养菌，以 PQQ 为辅基，以细胞色素 c 为天然电子受体，氧化甲醇生成甲醛并还原电子传递链；(2) NAD^+依赖性的甲醇脱氢酶存在于芽孢杆菌等革兰氏阳性菌中，催化甲醇氧化为甲醛的同时将 NAD^+还原为 NADH，进入呼吸链或用于合成代谢；(3)以氧气为电子受体的甲醇氧化酶主要存在于甲基营养型酵母中，该酶定位于过氧化物酶体，催化甲醇氧化生成甲醛并释放过氧化氢[22-25]。

随后，甲醛进一步被同化进入中心碳代谢的路径，根据中间产物的不同可分为两大类(图 1)。第一类为甲醛直接缩合的“磷酸糖途径”，代表性途径包括木酮糖单磷酸途径(xylulose monophosphate, XuMP)[26]和核酮糖单磷酸途径(ribulose monophosphate, RuMP)[24]。这类途径中甲醛直接与磷酸戊糖受体缩合，经裂解与重排生成 C3 代谢前体进入中心碳代谢途径[24,26]。第二类为通过甲酸/一碳载体的同化途径，代表性途径包括丝氨酸循环(serine cycle)[27]和还原甘氨酸途径(reductive glycine pathway, rGly)。在这类途径中，甲醛被进一步氧化为甲酸，甲酸与四氢叶酸(tetrahydrofolate, THF)结合生成活化的一碳单元(如 5,10-亚甲基-THF)，再通过羧化或转

表1 甲醇同化途径概述

Table 1 Overview of the methanol assimilation pathway

Substrate and pathway		Net reaction			References
		NAD(P)H	ATP	Carbon yield/%	
Natural C1 utilization pathway					
Methanol	RuMP pathway	5	1	66.7	[14]
	XuMP pathway	2	−1	66.7	[15]
	Serine pathway	−1	−3	100	[16]
	rGly pathway	2	−2	100	[17]
Synthetic C1 assimilation pathway					
Formaldehyde	GAA pathway	0	0	100	[18]
	SACA pathway	0	0	100	[19]
	EuMP pathway	2	1	66.7	[20]
Formaldehyde	FLS pathway	2	1	66.7	[13,21]

The production or consumption of reducing equivalents and ATP in each pathway was calculated using acetyl-CoA as the metabolic node. Positive values indicate net production, whereas negative values indicate net consumption. Carbon yield represents the fraction of carbon atoms from the C1 substrate that are incorporated into acetyl-CoA. It was calculated as follows: Carbon yield=(Number of carbon atoms in acetyl-CoA derived from the C1 substrate/Total number of carbon atoms in the C1 substrate participating in the reaction)×100%.

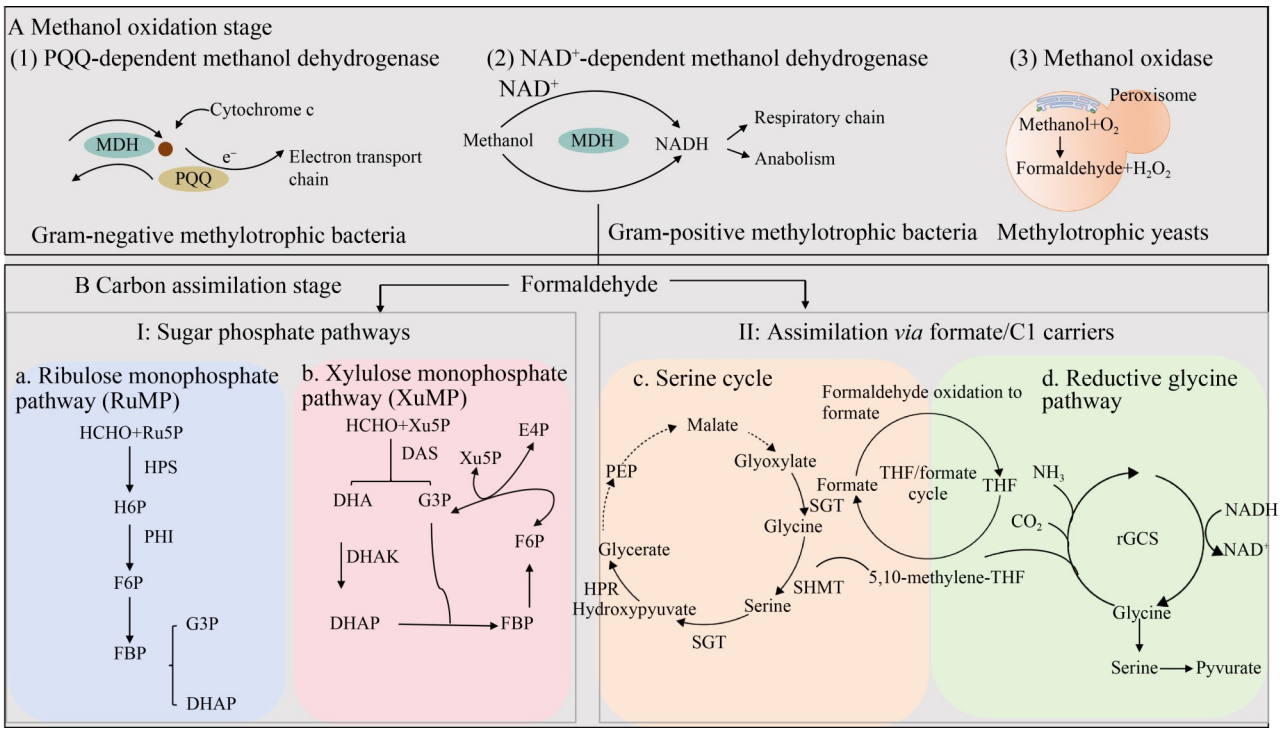

图1　天然有氧甲醇同化途径

Figure 1　Natural aerobic methanol assimilation pathways include methanol oxidation stage (A) and carbon assimilation stage (B). Methanol is first oxidized to formaldehyde by different methanol-oxidizing enzyme systems, which can be assimilated through distinct carbon fixation routes. PQQ-MDH: PQQ-dependent methanol dehydrogenase; NAD^+-MDH: NAD^+-dependent methanol dehydrogenase; Ru5P: Ribulose 5-phosphate; H6P: 3-hexulose-6-phosphate; F6P: Fructose 6-phosphate; FBP: Fructose 1,6-bisphosphate; G3P: Glyceraldehyde 3-phosphate; DHAP: Dihydroxyacetone phosphate; DHA: Dihydroxyacetone; Xu5P: Xylulose 5-phosphate; E4P: Erythrose 4-phosphate; THF: Tetrahydrofolate; 5,10-methylene-THF: 5,10-methylene-tetrahydrofolate; MDH: Methanol dehydrogenase; HPS: 3-hexulose-6-phosphate synthase; PHI: 6-phospho-3-hexuloisomerase; DAS: Dihydroxyacetone synthase; SHMT: Serine hydroxymethyltransferase; SGT: Serine-glyoxylate aminotransferase; MTK: Malate thiokinase; GCS: Reversed glycine cleavage system.

甲基反应整合到中心代谢[28-29]。

XuMP 途径主要存在于甲基营养型酵母和部分丝状真菌中[26]，RuMP 途径主要存在于多数甲基营养菌中[30-31]。在 RuMP 途径中，甲醛与核酮糖-5-磷酸(ribulose 5-phosphate, Ru5P)缩合，由 3-己酮糖-6-磷酸合酶(3-hexulose-6-phosphate synthase, HPS)催化生成 C6 中间体己酮糖-6-磷酸(3-hexulose-6-phosphate, H6P)，随后经 6-磷酸-3-己酮糖异构酶(6-phospho-3-hexuloisomerase, PHI)转化为果糖-6-磷酸(fructose 6-phosphate, F6P)[24]；在 XuMP 途径中，甲醛与木酮糖-5-磷酸(xylulose 5-phosphate, Xu5P)缩合，生成 C6 中间体，再经异构化为 F6P。F6P 进一步通过糖酵解及磷酸戊糖途径的逆反应重组，生成糖酵解前体甘油醛-3-磷酸(glyceraldehyde 3-phosphate, G3P)和二羟基丙酮磷酸(dihydroxyacetone phosphate, DHAP)；最后通过非氧化性磷酸戊糖途径将剩余碳骨架重新组装为 Ru5P 或 Xu5P，实现甲醛受体的循环再生[24]。RuMP 和 XuMP 途径中甲醇氧化步骤的酶学差异导致不同的能

量效率[22,24]。XuMP 途径使用醇氧化酶(alcohol oxidase, AOX)，产生 H_2O_2 并耗散还原当量；而 RuMP 途径中的甲醇氧化模块有多样性：NAD^+ 依赖型甲醇脱氢酶(methanol dehydrogenase, MDH)产生 NADH，实现还原力的高效捕获与利用；而 PQQ 依赖型的 MDH 通过细胞色素等电子传递组分进入呼吸链[23]。

丝氨酸循环主要存在于部分甲基营养菌及兼性甲基营养菌中，最初在 *M. extorquens* 中被发现，是 α-变形菌普遍采用的甲醛/甲酸同化途径[27]。与 RuMP 和 XuMP 途径通过缩合反应直接固定甲醛不同，在丝氨酸循环途径中，甲醛进一步被氧化为甲酸，甲酸在甲酰-THF 合成酶(formate-THF ligase, FtfL)催化下消耗 ATP 生成 10-甲酰-THF；随后经 2 次还原反应转化为 5,10-亚甲基-THF，进而通过丝氨酸羟甲基转移酶(serine hydroxymethyltransferase, SHMT)将 5,10-亚甲基-THF 的一碳单元转移至甘氨酸，生成丝氨酸，丝氨酸经多步反应转化为丙酮酸和乙酰辅酶 A[32]。该途径每合成一个乙酰辅酶 A 需要 1 分子 NAD(P)H 及 3 分子 ATP[33]，能量效率明显低于 RuMP 途径(表 1)，但该途径具有较高的碳原子经济性[34]。

还原甘氨酸途径是近年来受到广泛关注的新型碳一同化路线，最初在脱硫弧菌(*Desulfovibrio desulfuricans*)等少数细菌中被发现[28-29]。与上述循环途径不同，rGly 途径呈线性结构，以甘氨酸裂解系统(glycine cleavage system, GCS)为代谢枢纽，在碳原子经济性上表现出显著优势；该途径的核心是逆向运行甘氨酸裂解系统，以 5,10-亚甲基-THF、CO_2 和 NH_3 为底物，在 GCS 催化下合成甘氨酸，生成的甘氨酸再经 SHMT 催化结合另一个 5,10-亚甲基-THF 生成丝氨酸，最后丝氨酸通过脱水或转氨反应转化为丙酮酸，从而接入中心碳代谢(图 1)[28]。该途径可利用甲醇或甲酸作为初始碳源，整体反应线性、无需复杂的循环平衡，且所需 ATP 较少[28]。该途径凭借其线性代谢路径优势，已在 *E. coli* 及 *S. cerevisiae* 在内的多种模式宿主中成功实现异源重构，并验证了其利用碳一原料进行生物转化的功能活性[35-36]。

1.2 厌氧甲醇同化途径

与有氧条件下的氧化磷酸化不同，厌氧环境中微生物无法利用氧气作为电子受体，因此甲醇的代谢必须依赖其他替代性电子受体，如 CO_2 等[37-41]。根据终端电子受体及代谢产物的差异，厌氧利用甲醇的微生物主要分为产甲烷菌与产酸菌(以产乙酸菌为代表)两大类[37-41]。甲基营养型产甲烷古菌，如 *Methanolobus*、*Methanomethylovorans* 和 *Methanosarcina*，能够直接利用甲醇作为底物生成甲烷[37]。该过程通过 MtaABC 甲基转移酶复合物催化甲醇与辅酶 M (CoM)生成甲基辅酶 M (CH_3-S-CoM)，随后在甲基辅酶 M 还原酶(methyl-coenzyme M reductase, MCR)催化下还原为 CH_4[37]。该过程伴随电子传递链生成跨膜质子梯度驱动 ATP 合成。产酸细菌，如伍氏醋杆状菌(*Acetobacterium woodii*)、*B. methylotrophicum* 等[38-40]，则通过另一条途径将甲醇转化为乙酸、丁酸等有机酸[40-41]。与产甲烷菌类似，产酸菌通过 MtaABC 甲基转移酶复合物催化甲醇与 THF 生成甲基四氢叶酸(CH_3-THF)，随后进入 Wood-Ljungdahl 途径的甲基支路，还原形成亚甲基-THF；与此同时，CO_2 通过该途径的羰基支路被还原为一氧化碳(CO)[39-41]。最终，甲基支路与羰基支路的产物在乙酰辅酶 A 合酶(acetyl-CoA synthase, ACS)催化下缩合生成乙酰辅酶 A[39] (图 2)。

区别于好氧甲醇代谢过程中必然经历毒性中间体甲醛的积累，厌氧甲醇代谢途径通过甲基转移反应直接将甲醇的甲基转移到高亲和力受体(CoM 或 THF)，从而有效规避了游离甲醛的生成[37-40]。这一代谢特性赋予了厌氧菌株显著更高的甲醇耐受性，使其能够在高浓度甲醇环境中维持正常代谢[38-40]。同时，厌氧条件下无需供氧也降低了发酵过程中的能耗与安全风险[38-43]。因此，近年来以产乙酸菌为代表的厌

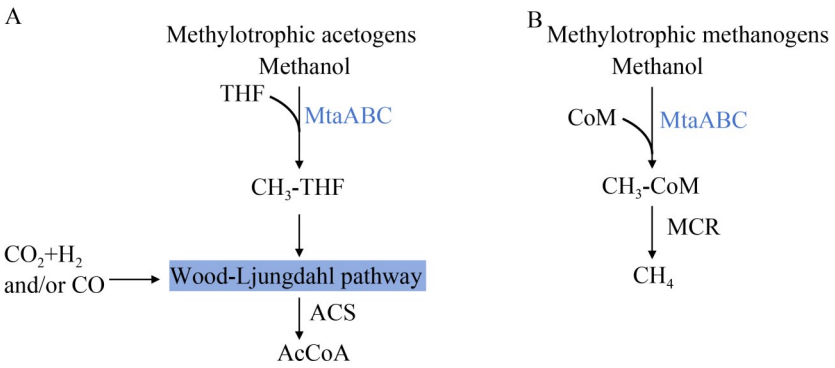

图2 天然厌氧甲醇同化途径

Figure 2 Natural anaerobic methanol assimilation pathways. A: Acetogen; B: Methanogen. MtaABC: Methanol methyltransferase system; ACS: Acetyl-CoA synthase; MCR: Methyl-coenzyme M reductase; AcCoA: Acetyl coenzyme A.

氧甲醇生物转化体系在利用甲醇生产乙酸、乙醇、丁酸等化学品方面受到越来越多的关注，成为甲醇生物炼制领域的重要研究方向之一[42-43]。

2 人工甲醇同化途径

开发具有高碳原子经济性与能量利用效率的人工甲醇同化途径，是当前代谢工程的研究重点[13]。通过理性构建非天然代谢网络，在简化转化步骤的同时，精准调控 ATP 与还原力供给，从而突破理论碳得率限制，实现更高效的甲醇同化。当前基于模块化设计与代谢重构策略，已成功构建多条具有较高碳转化效率的人工合成途径(图 3)[18]。

2.1 EuMP 途径

甲醇在细胞内通常先被氧化生成甲醛，随后进入中心代谢网络的核心节点[20,22-25]。Wu 等[20]设计并构建了赤藓酮糖单磷酸(erythrulose monophosphate pathway, EuMP)的甲醛人工同化途径(图 3)，该途径基于一种新型醛缩酶，催化二羟基丙酮磷酸生成赤藓糖-4-磷酸。EuMP 途径在前体代谢物合成的能量效率接近 RuMP 途径，优于 XuMP 途径和丝氨酸循环[20]。每生成一分子乙酰辅酶 A，EuMP 途径净生成 2 分子 NAD(P)H 和 1 分子 ATP，具备更优的能量经济性[20]。

2.2 GAA 途径

Yang 等[18]设计出羟乙醛同化途径(glycolaldehyde assimilation pathway, GAA)，通过磷酸酮醇激酶等将甲醛转化为乙酰辅酶 A (图 3)，该途径碳原子转化效率可达 100%；相较于已知的天然途径，GAA 途径展现出更优的原子经济性；该途径不额外消耗 ATP 及 NAD(P)H，具备较高的能量经济性。

2.3 FLS 途径

Siegel 等[13]设计了甲醛裂合酶(formolase, FLS)，催化 3 分子甲醛缩合生成 1 分子二羟基丙酮(dihydroxyacetone, DHA)，随后被二羟基丙酮激酶磷酸化为二羟基丙酮磷酸(DHAP)进入中心代谢，此路径被称为 FLS 途径(图 3)。与天然甲酸利用途径相比，该路径反应步骤少、碳利用效率高[13]。在此基础上，Cai 等[44]将 FLS 途径与下游反应模块整合，构建了一条以 CO_2 为原料合成淀粉的人工路径。该研究为实现 CO_2 的生物制造提供了重要的理论验证，并开拓了新的技术路径[44]。

2.4 SACA 途径

Lu 等[19]构建了乙酰辅酶 A 合成途径(synthetic acetyl-CoA pathway, SACA)，将 2 分子甲醛转化为 1 分子乙酰辅酶 A (图 3)。该途径基

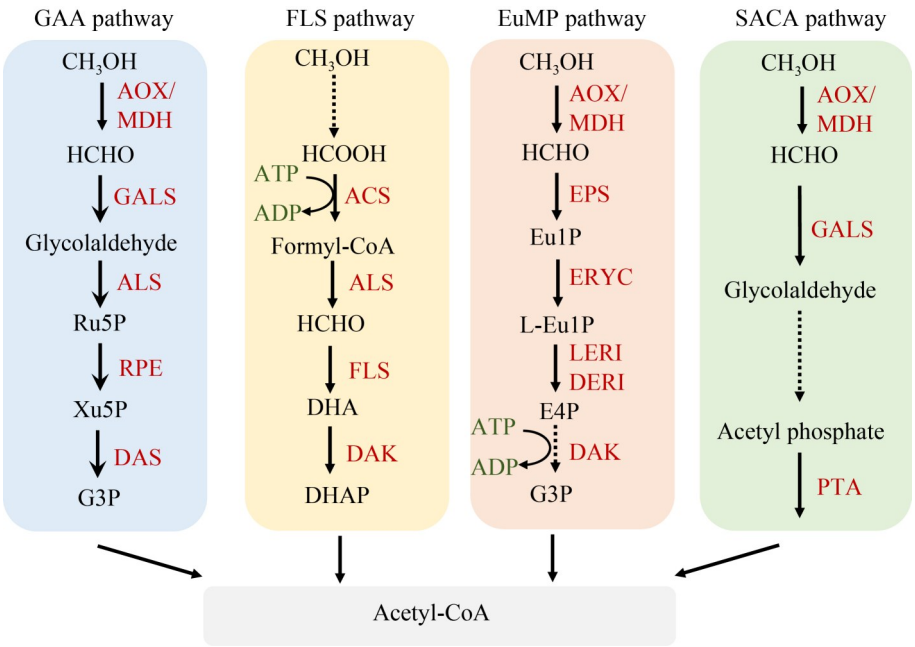

图3　人工甲醇同化途径

Figure 3　Synthetic methanol assimilation pathways. HCHO: Formaldehyde; HCOOH: Formic acid; Ru5P: ribulose 5-phosphate; Xu5P: xylulose 5-phosphate; G3P: glyceraldehyde 3-phosphate; DHA: Dihydroxyacetone; DHAP: Dihydroxyacetone phosphate; Eu1P: Erythrulose 1-phosphate; L-Eu1P: L-erythrulose 1-phosphate; E4P: Erythrose 4-phosphate; GALS: Glycolaldehyde synthase; ALS: Aldolase; RPE: Ribulose-5-phosphate 3-epimerase; DAS: Dihydroxyacetone synthase; ACS: Acetyl-CoA synthetase; FLS: Formolase; DAK: Dihydroxyacetone kinase; EPS: Erythrulose 1-phosphate synthase; ERYC: Erythrulose 1-phosphate epimerase; LERI: L-erythrulose 1-phosphate isomerase; DERI: D-erythrose 4-phosphate isomerase; PTA: Phosphotransacetylase; ACPS: Acetyl-phosphate synthase.

于 2 步核心反应：首先利用羟乙醛合酶(glycolaldehyde synthase, GALS)催化甲醛缩合生成羟乙醛；随后通过乙酰磷酸合酶(acetyl-phosphate synthase, ACPS)与磷酸乙酰转移酶(phosphate acetyltransferase, PTA)进一步转化为乙酰辅酶 A。SACA 途径为目前已知唯一以甲醛为专一底物合成乙酰辅酶 A 的碳一同化途径；该途径和天然代谢网络不重叠、不依赖 ATP、碳守恒且对氧气不敏感(表 1)[19]。因此在目标产物的高效合成方面展现出独特优势[19]。

3　甲醇利用菌株研究进展

3.1　天然甲醇利用菌株

自然界中存在两大类甲醇利用菌株：以 *B. methanolicus* 和 *M. extorquens* 为代表的革兰氏阳性菌和革兰氏阴性菌，以及以 *K. phaffii* (旧称 *Pichia pastoris*)和 *O. polymorpha* 为代表的甲基营养型酵母[4,23-26,33]。这些微生物均拥有完整的甲醇代谢途径，能够实现以甲醇为唯一碳源和能源生长[4,23-26,33]。

B. methanolicus 是一种质粒依赖型甲醇利用菌株[25]。该菌的甲醇代谢依赖于 NAD^+依赖型甲醇脱氢酶(MDH)和核酮糖单磷酸(RuMP)途径[24-25]。理论计算表明，该代谢途径消耗 3 分子甲醇合成 1 分子丙酮酸，同时生成 4 mol NAD(P)H 及 1 mol ATP[4]。凭借其独特的遗传背景与生理特性，该菌株被视为极具潜力的甲醇生物转化底盘细胞[4,24-25]。

M. extorquens 属于 α-变形菌，其甲醇代谢以丝氨酸循环为核心同化途径，主要包含 2 个功能模块，依赖特定金属辅因子(钙或镧系元素)的甲醇脱氢酶(MDH)催化甲醇氧化为甲醛；甲醛经四氢叶酸(THF)途径激活，形成亚甲基四氢叶酸(CH_2=THF)，随后进入丝氨酸循环，最终生成中心代谢物乙酰辅酶 A[23]。基于其清晰、可预测的代谢网络，*M. extorquens* 已成为甲醇合成高附加值化学品的模式菌株与研究平台[23]。然而，该菌株存在生长速率缓慢、碳转化效率较低以及固有的代谢网络复杂等问题，这些因素共同制约了其作为高效甲醇生物转化平台的开发与应用[45-46]。

O. polymorpha 与 *K. phaffii* 均为甲醇利用的关键模式菌株，以木酮糖单磷酸途径(XuMP)为核心同化路径[47]。甲醇首先在过氧化物酶体内被醇氧化酶氧化为甲醛；随后甲醛与木酮糖-5-磷酸(xylulose-5-phosphate, Xu5P)在二羟基丙酮合酶(dihydroxyacetone synthase, DAS)的催化下生成甘油醛-3-磷酸(glyceraldehyde-3-phosphate, G3P)和二羟基丙酮(dihydroxyacetone, DHA)[26]。DHA 被二羟基丙酮激酶转化为二羟基丙酮磷酸(DHAP)进入中心碳代谢[26]。*O. polymorpha* 和 *K. phaffii* 能够利用成分简单的廉价合成培养基进行高密度发酵，并拥有强效且严格受甲醇诱导的启动子[48]。随着 CRISPR/Cas9 等先进基因编辑工具的成熟与应用，*O. polymorpha* 已被成功改造为高效利用甲醇合成高附加值化学品的细胞工厂[48-50]。*K. phaffii* 具备高效的甲醇代谢能力、较广的 pH 耐受范围，且在工业重组蛋白生产领域积累了广泛的应用基础，因此被视为构建甲醇生物转化微生物细胞工厂的理想宿主[51-54]。

3.2 非天然甲醇利用菌株

人工合成甲醇利用细胞工厂的构建通常借鉴天然甲醇利用菌株的甲醇转化与代谢机制，运用合成生物学与代谢工程手段对底盘细胞进行系统性改造[4]。模式底盘细胞包括 *E. coli*、解脂耶氏酵母(*Yarrowia lipolytica*)和 *S. cerevisiae* 等(表 2)。其遗传背景清晰、遗传操作工具丰富、生理特性明确，为成功创制人工合成甲基营养细胞工厂提供了坚实基础[4]。

S. cerevisiae 凭借其出色的环境适应性与代谢可塑性，被视为构建人工甲基营养微生物的理想底盘细胞之一[65]。其具备固有的区室化特性，能有效隔离有毒代谢中间体并促进酶底物浓度的升高和代谢通道效应，从而在实现复杂代谢途径重构方面展现出独特优势[65]。*S. cerevisiae* 拥有还原甘氨酸途径(rGly)的完整酶系，理论上可催化甲酸同化，但因内源关键酶线粒体 C1-四氢叶酸合成酶(mitochondrial C1-tetrahydrofolate synthase, MIS1)和/或甘氨酸裂解系统(GCS)活性低，不足以支持所需的代谢通量[36]。Zhan 等[65]结合模块化设计与适应性实验室进化(adaptive laboratory evolution, ALE)策略，成功实现 *S. cerevisiae* 以甲醇为唯一碳源生长。然而，*S. cerevisiae* 利用甲醇仍面临细胞对甲醇及甲醛的耐受性有限，以及碳一代谢途径中关键酶的催化效率较低等瓶颈[65]。

Y. lipolytica 是工业生产中常用的底盘微生物，也是研究最多的非常规酵母之一[66]。该酵母具备可通过工程化改造获得利用多种碳源的能力，展现出显著的代谢可塑性；相较于 *S. cerevisiae*，*Y. lipolytica* 表现出更强的有机溶剂耐受性[66-67]。其独特的代谢可塑性和强大的抗逆性使其成为生产多种化学品的通用平台[66]。此外，*Y. lipolytica* 能形成大量的过氧化物酶体完成脂质氧化，为重构甲醇代谢途径提供了独特的代谢改造基础[66]。Wang 等[61]通过在 *Y. lipolytica* 中构建细菌 RuMP 途径和部分 *K. phaffii* 的 XuMP 途径组成的嵌合甲醇同化通路，并结合 ALE 策略，使工程菌在以甲醇为唯一碳源的条件下能维持细胞活性；进一步揭示了 *Y. lipolytica* 具有利用甲醇作为共底物的潜力，甲醇可进入蛋白源氨基酸等生物质组分，并为细胞维持提供还原力和能量支持，为进一步构建合成甲基营养型 *Y. lipolytica* 细胞工厂，并探索其在甲醇衍生产物合成中的应用奠定了基础。

表2　人工合成甲醇利用菌

Table 2　Engineering synthetic methylotrophic microbes

Assimilation pathway	Chassis	Engineering strategy	Doubling time/h	Methanol consumption rate/[g/(L·h)]	Advantages and limitations relative to natural methylotrophs	References
RuMP pathway	*E. coli*	Dynamic copy-number tuning of RuMP pathway genes; introduction of an evolved *mdh* gene; ALE; restoration of wild-type *mutS*	3.5	–	Advantages: the engineered strain grows at a rate comparable to, or faster than, several model natural methylotrophs Limitations: methanol oxidation and formaldehyde assimilation require finely tuned pathway balancing, making strain construction complex	[55]
	C. glutamicum	Expression of heterologous *xylA*, *mdh*, *hps*, and *phi*; deletion of *rpiB*, *adhE*, and *ald*; ALE	15.8	–	Advantages: introducing the RuMP cycle into an amino-acid-producing chassis links methanol assimilation with amino acid metabolism Limitations: growth remains dependent on a co-substrate, and methanol assimilation is weaker than in natural methylotrophs	[56]
	Bacillus subtilis	Construction of an Mdh-Hps-Phi self-assembling multienzyme complex using SpyTag/SpyCatcher and DogTag/DogCatcher; deletion of the formaldehyde dehydrogenase-related genes *fdhA*, *adhB*, and the 6-phosphogluconate dehydrogenase gene *gnd*; overexpression of the fructose-1,6-bisphosphatase gene *glpX*, transaldolase gene *tal*, and phosphoglycerate kinase gene *pgk*	–	0.129	Advantages: scaffold-assisted assembly of Mdh, Hps, and Phi improves methanol conversion through the RuMP cycle Limitations: rapid growth on methanol as the sole carbon source has not been achieved	[57]
XuMP pathway	*E. coli*	Expression of the *mdh* and *das*; deletion of the formaldehyde detoxification-related genes *frmA*/*frmB*, phosphofructokinase genes *pfkA*/*pfkB*, and the TCA cycle gene *sucA*; downregulation of the glyceraldehyde-3-phosphate dehydrogenase gene *gapA*; ALE	–	0.034	Advantages: the hybrid Mdh-Das pathway provides an alternative methanol assimilation architecture distinct from the canonical RuMP cycle Limitations: growth still requires a nutrient co-substrate, and methanol-only growth has not been achieved	[58]
	S. cerevisiae	Integration of the alcohol oxidase gene *aox*, catalase gene *cat*, dihydroxyacetone synthase gene *das2*, and dihydroxyacetone kinase gene *dak* into the *S. cerevisiae* chromosome	–	0.033	Advantages: a *K. phaffii*-derived XuMP module provides a starting point for engineering methanol metabolism in *S. cerevisiae* Limitations: improvements in methanol consumption and growth remain modest compared with natural XuMP-dependent methylotrophic yeasts	[59]

(待续)

(续表2)

Assimilation pathway	Chassis	Engineering strategy	Doubling time/h	Methanol consumption rate/[g/(L·h)]	Advantages and limitations relative to natural methylotrophs	References
XuMP/ RuMP pathway	*Y. lipolytica*	Expression of the methanol dehydrogenase gene *mdh*, 3-hexulose-6-phosphate synthase gene *hps*, 6-phospho-3-hexuloisomerase gene *phi*, dihydroxyacetone synthase gene *pdas1*, dihydroxyacetone kinase gene *dak2*; deletion of the formaldehyde dehydrogenase gene *fld1*; overexpression of the Ru5P/ Xu5P-regeneration genes *tkl1*, *pfk*, *fba*, *rpe1*, and *BmglpX*(*P*); ALE	–	0.015	Advantages: engineering RuMP/ XuMP-related modules extends the potential of oleaginous yeasts for methanol-based biomanufacturing Limitations: growth on methanol as the sole carbon source has not been demonstrated	[60-61]
	K. phaffii	Overexpression of the E4P phosphatase gene *yidA* and erythrose reductase gene *alr*; deletion of the sedoheptulose-1,7-bisphosphatase gene *shb*; introduction of the bacterial RuMP pathway fusion gene *hpsi*; overexpression of TPI and RPE1	–	–	Advantages: rewiring native methylotrophy redirects methanol-derived carbon towards target product synthesis Limitations: the strategy is product-oriented and does not primarily improve general growth on methanol	[15]
rGly pathway	*K. phaffii*	Deletion of the dihydroxyacetone synthase genes *das1* and *das2*; overexpression of the rGlyP glycine synthesis module genes *mis1*, *gcv1*, *gcv2*, and *gcv3*; targeting of the methanol oxidation module AOX-FLD-FGH to peroxisomes; overexpression of *shm1*	36	0.064	Advantages: introducing the MFORG pathway enables co-assimilation of methanol or formate with CO_2 in a natural methylotrophic yeast Limitations: growth remains weaker than that supported by the native XuMP pathway	[62]
	S. cerevisiae	Expression of the peroxisome-targeted AOX, FLD, FGH, and FDH methanol/formate oxidation module; expression of *mis1*, *gcv1*, *gcv2*, and *gcv3* to construct the rGlyP glycine synthesis module; overexpression of *shm1*	–	0.020	Advantages: the MFORG pathway extends methanol/ formate and CO_2 co-assimilation to a non-methylotrophic eukaryotic chassis Limitations: methanol consumption remains low, and pathway coordination is less efficient than in natural methylotrophic yeasts	[62]
	Pseudomonas putida	Expression of the C1 module genes *fhs*, f*chA*, and *folD*; expression of a methanol dehydrogenase gene and the formaldehyde dehydrogenase gene *fdhA*; deletions of gcvTPH-I/II, serA, and PP_2533	10.2	–	Advantages: growth-coupled engineering shows that methanol-derived carbon can enter rGlyP-linked C1 metabolism in a robust bacterial chassis Limitations: the study remains a proof of concept and does not establish complete methylotrophic growth	[63]

(待续)

(续表2)

Assimilation pathway	Chassis	Engineering strategy	Doubling time/h	Methanol consumption rate/[g/(L·h)]	Advantages and limitations relative to natural methylotrophs	References
EuMP pathway	*E. coli*	Overexpression of the DHAP-dependent aldolase gene *fucA* or *rhaD*; expression of D-erythrulose 1-phosphate 3-epimerase gene *eryC*, L-erythrulose 1-phosphate isomerase gene *lerI*, and D-erythrulose 4-phosphate isomerase gene *derI*	–	–	Advantages: the EuMP cycle provides an energy-efficient formaldehyde assimilation route with theoretical performance comparable to the RuMP cycle Limitations: current work mainly demonstrates formaldehyde assimilation, rather than direct methanol utilization	[20]
Serine cycle	*Pseudomonas putida*	Genomic integration of *ftfL-mtdA-fch* in a Δ*serA* ΔΔ*gcvTHP* background; overexpression of *ltaE* and *yiaY*; deletion of *thiO*, *lapA*, and *lapF*; expression of *E. coli ltaE* C188Y	3.2	–	Advantages: the synthetic serine cycle can exploit endogenous PQQ-dependent methanol oxidation and is therefore well matched to *P. putida* metabolism Limitations: growth still requires glucose as a co-substrate, and methanol-dependent growth comparable to natural methylotrophs remains to be achieved	[64]

E. coli 是经典的原核模式微生物，在工业应用中有巨大潜力[68]。通过合成生物学手段引入高效的甲醇同化模块，可将其底物利用范围拓展至甲醇等碳一原料[68]。Chen 等[69]率先在 *E. coli* 中成功引入了核酮糖单磷酸(RuMP)途径，并结合 ALE 策略，获得的工程菌株可以甲醇为唯一碳源高效生长。Keller 等[70]通过连续恒化培养结合适应性进化，最终获得了能以甲醇为唯一碳源和能源的合成甲基营养型菌株，其倍增时间为 8 h，达到与部分天然甲基营养菌相近的水平。Nieh 等[55]通过进化工程结合动态拷贝数调控策略成功构建出以甲醇为唯一碳源的 *E. coli*，倍增时间仅 3.5 h，已与 *M. extorquens* 和 *B. methanolicus* 等部分天然甲基营养菌相当甚至更快。

综上所述，构建非天然甲基营养菌的策略主要聚焦于 2 个方向：(1)对模式菌株进行代谢重编程，提高甲醇同化途径碳通量；(2)通过适应性实验室进化筛选获得具备甲基营养表型的菌株。尽管上述策略已初步实现了工程菌株对碳一原料的利用，但总体而言，同化效率仍需进一步提高。

4 甲醇作为碳源合成高附加值化学品

面对日益严峻的化石燃料危机与不断加剧的环境压力，开发基于可再生原料的高附加值化学品可持续生产路线已成为当前生物制造领域的研究热点[1-2]。甲醇来源广泛、成本低廉，展现出替代传统糖基原料的巨大潜力[3,33]。甲醇作为碳源已成功合成多个产品，例如有机酸、脂肪酸、氨基酸、单细胞蛋白及生物医药单体等(表 3)。这些进展不仅丰富了甲醇生物转化的产品谱系，也为构建非粮碳源驱动的绿色制造体系奠定了重要基础[33,46]。

4.1 有机酸

有机酸作为合成多种精细化学品的关键前体，在食品、制药及化工等领域具有广泛的应用[33]。其中，以甲醇为原料合成有机酸是实现可持续生物制造的重要策略[33]。研究者通过对甲基营养菌进行代谢工程改造，已成功实现了多种有机酸的合成[46]。

丙酮酸作为微生物碳代谢网络中的核心中间体，是合成多种高附加值化学品的重要前体，

表3 甲醇作为碳源合成高附加值化学品研究进展

Table 3 Research advances in chemical synthesis using methanol as the carbon source

Product	Titer/(g/L)	Chassis	Engineering strategy	Fermentation mode	Carbon source	Yield/(g/g)	References
Pyruvic acid	0.26	*S. cerevisiae*	Expression of alcohol oxidase, catalase, dihydroxyacetone synthase 2, and dihydroxyacetone kinase	Shake-flask fermentation	Methanol as the sole carbon source	0.25	[59]
D-lactic acid	3.48	*K. phaffii*	Overexpression of D-lactate dehydrogenase	Test-tube fermentation	Methanol as the sole carbon source	0.22	[71]
L-lactic acid	4.20	*K. phaffii*	Engineering the cofactor preference of lactate dehydrogenase, blocking L-lactate consumption, and constructing dual biosynthetic pathways in the cytosol and mitochondria	Fed-batch fermentation	Methanol as the sole carbon source	0.018	[72]
	17.00	*K. phaffii*	Overexpression of the L-lactate dehydrogenase LdhL; deletion of L-lactate cytochrome-c oxidoreductase gene *cyb2*; and overexpression of the transcriptional activators genes *mxr1* and *mit1*	Fed-batch fermentation	Methanol as the sole carbon source during the production phase; glycerol for initial biomass accumulation	–	[73]
	25.00	*O. polymorpha*	Optimization of L-lactate dehydrogenase *Pa*Ldh expression, engineering of NADPH cofactor preference, and mitochondrial compartmentalization	Fed-batch fermentation	Methanol as the sole carbon source	0.22	[8]
	0.21	*K. phaffii*	Construction of the MFORG pathway; deletion of the dihydroxyacetone synthase genes *das1* and *das2*; reconstruction of methanol oxidation, formate oxidation, glycine synthesis, and pyruvate synthesis modules; targeting of the methanol oxidation module to peroxisomes; and further overexpression of lactate dehydrogenase (LDH)	Shake-flask fermentation	Methanol plus CO_2/bicarbonate co-assimilation	–	[62]
	0.07	*S. cerevisiae*	Introduction of the MFORG pathway and overexpression of LDH	Shake-flask fermentation	Methanol plus CO_2/bicarbonate co-assimilation	–	[62]

(待续)

(续表3)

Product	Titer/(g/L)	Chassis	Engineering strategy	Fermentation mode	Carbon source	Yield/(g/g)	References
3-hydroxypropionic acid	21.40	*K. phaffii*	Overexpression of aspartate 1-decarboxylase, β-alanine-pyruvate aminotransferase, and 3-hydroxypropionate dehydrogenase; increasing the gene copy number of aspartate 1-decarboxylase and attenuating the expression of the 3-hydroxypropionate dehydrogenase gene	Fed-batch fermentation	Methanol as the sole carbon source during the production phase; glycerol for initial biomass accumulation	0.15	[74]
	48.20	*K. phaffii*	Optimization of malonyl-CoA reductase (MCR) expression, enhancement of malonyl-CoA and NADPH supply, and downregulation of the methanol dissimilation pathway	Fed-batch fermentation	Methanol as the sole carbon source	0.23	[75]
	27.00	*K. phaffii*	Construction of the β-alanine pathway, enhancement of NADPH and precursor supply, and overexpression of a monocarboxylate permease and a lactate-proton symporter	Fed-batch fermentation	Methanol as the sole carbon source during the production phase; glycerol for initial biomass accumulation	0.19	[76]
	7.10	*O. polymorpha*	Expression of malonyl-CoA reductase (MCR), enhancement of acetyl-CoA and malonyl-CoA precursor supply, and reinforcement of NADPH supply	Fed-batch fermentation in shake flasks	Methanol as the sole carbon source during the production phase	0.14	[77]
Malic acid	2.79	*K. phaffii*	Construction of a reductive TCA-based malate synthesis module; expression of a C4-dicarboxylate transporter; deletion of *gpi* to redirect methanol-derived carbon flux from the XuMP cycle	Methanol-fed shake-flask cultivation	Methanol as the major carbon source during the production phase	–	[78]
	13.20	*O. polymorpha*	Overexpression of pyruvate carboxylase, malate dehydrogenase, and a malate transporter to enhance malate biosynthesis and export	Methanol-fed shake-flask cultivation	Methanol as the sole carbon source during the production phase	–	[79]
Succinic acid	0.92	*Y. lipolytica*	Construction of methanol assimilation and Xu5P regeneration pathways, overexpression of Hsp70, and deletion of succinate dehydrogenase subunit 5	Shake-flask cultivation	Methanol as the sole carbon source during the production phase	0.15	[80]

(待续)

(续表3)

Product	Titer/(g/L)	Chassis	Engineering strategy	Fermentation mode	Carbon source	Yield/(g/g)	References
Fatty acids	23.4	*K. phaffii*	Blocking fatty acid reactivation, enhancing acetyl-CoA and NADPH supply, and reinforcing methanol assimilation	Fed-batch fermentation	Methanol as the sole carbon source	0.078	[81]
Fatty alcohols	2	*K. phaffii*	Expression of fatty acyl-CoA reductase in a fatty-acid-overproducing chassis	Fed-batch fermentation	Methanol as the sole carbon source	0.008	[81]
Fatty acids	15.9	*O. polymorpha*	Deletion of the fatty acyl-CoA synthetase gene *faa1*; adaptive laboratory evolution and inactivation of the putative lipase gene *lpl1* and the zinc metabolism-related membrane protein gene *izh3* to improve methanol tolerance; simultaneous enhancement of Xu5P, NADPH, and acetyl-CoA supply	Fed-batch fermentation	Methanol as the sole carbon source	0.12	[82]
β-alanine	5.6	*K. phaffii*	Overexpression of L-aspartate-α-decarboxylases, increasing ADC copy number, and enhancing aspartate supply *via* aspartate dehydrogenase	Two-stage fermentation	Methanol as the sole carbon source during the production phase; glycerol for initial biomass accumulation	–	[83]
Single-cell protein	32.1	*K. phaffii*	ALE; overexpression of glutamine synthetase; and deletion of genes involved in cell wall biosynthesis to increase protein	Fed-batch fermentation	Methanol as the sole carbon source during the production phase; glycerol for initial biomass accumulation	–	[84]
Monacolin J	0.594	*K. phaffii*	Construction of pathways associated with the lovastatin biosynthetic gene cluster and pathway division-based co-cultivation to improve production	Co-culture fermentation	Methanol as the sole carbon source during the production phase; glycerol for initial biomass accumulation	–	[85]
Lovastatin	0.251	*K. phaffii*	Expression of key enzymes involved in lovastatin biosynthesis and pathway division-based co-cultivation to alleviate metabolic burden	Co-culture fermentation	Methanol as the sole carbon source during the production phase; glycerol for initial biomass accumulation	–	[85]
Chondroitin sulfate	2.1	*K. phaffii*	Construction of the chondroitin biosynthetic pathway, optimization of key enzyme expression, and expression of chondroitin 4-O-sulfotransferase; PAPS supply enhancement	Fed-batch fermentation	Methanol as the sole carbon source during the production phase; glycerol for initial biomass accumulation	–	[86]

(待续)

(续表3)

Product	Titer/(g/L)	Chassis	Engineering strategy	Fermentation mode	Carbon source	Yield/(g/g)	References
Catharanthine	0.002 57	*K. phaffii*	Construction and optimization of the catharanthine biosynthetic pathway, P450/redox and SAM-supply engineering, and blocking competing pathways	Fed-batch fermentation	Methanol plus mannitol during production	–	[87]
α-humulene	1.65	*M. extorquens*	Expression of α-humulene synthase and farnesyl diphosphate synthase; introduction of the mevalonate pathway and optimization of ribosome-binding sites; using a carotenoid-deficient strain	Fed-batch fermentation	Methanol as the sole carbon source	0.031	[88]

在工业生物制造中具有广泛应用[89]。通过在 *S. cerevisiae* 中导入 *K. phaffii* 的甲醇利用途径，实现了甲醇生物合成丙酮酸，摇瓶水平产量达到 0.26 g/L[59]。仍需通过系统的代谢工程策略进一步优化，提升产量[89]。

乳酸(2-羟基丙酸)是一种重要的平台化学品[8]。Guo 等[62]将甲醇/甲酸氧化模块与 rGly 途径耦合，并在 *K. phaffii* 和 *S. cerevisiae* 中实现甲醇或甲酸与 CO_2 的协同利用，成功合成乳酸，产量分别为 0.21 g/L 和 0.07 g/L。Yamada 等[71]将来源于肠系膜明串珠菌的乳酸脱氢酶 D-Ldh 在甲基营养酵母 *K. phaffii* 中表达，D-乳酸产量达到 3.48 g/L。Bachleitner 等[73]进一步通过共过表达转录调控因子 Mxr1 与 Mit1 以强化甲醇利用途径，使 *K. phaffii* 的乳酸产量提升至 17 g/L。然而，该水平仍显著低于传统工业菌株利用 C5/C6 糖底物的产量 150 g/L[73]。因此，如何在甲基营养宿主中系统优化代谢途径进一步提升乳酸产量，仍是当前需要解决的关键问题。

3-羟基丙酸(3-hydroxypropionic acid, 3-HP)是一种重要的平台化合物，其生物合成途径主要包括甘油途径、丙二酰辅酶 A 途径及 β-丙氨酸途径[90]。在以甲醇为碳源的体系中，*K. phaffii* 通过丙二酰辅酶 A 途径产量达 48.2 g/L，而经 β-丙氨酸途径也可达到 21.4 g/L[74-75]。相比之下，*O. polymorpha* 在强化丙二酰辅酶 A 途径并提升辅因子供应后，3-HP 产量为 7.1 g/L[77]。尽管该结果已有明显提升，但其产量仍显著低于 *K. phaffii* 所达到的水平。

苹果酸(malic acid, MA)[91-93]与琥珀酸(succinic acid, SA)均为具有重要工业价值的 C4-二元羧酸，广泛应用于食品、饲料及化工领域[94-95]。在 *O. polymorpha* 中，通过引入高效苹果酸转运蛋白并结合发酵策略优化，在甲醇培养基中实现了苹果酸产量 13.2 g/L[79]。Zhang 等[80]通过构建合成甲基营养型 *Y. lipolytica*，并敲除琥珀酸脱氢酶及优化氮源等策略，利用甲醇实现了琥珀酸产量 0.92 g/L。相比之下，利用甘油为底物的工程化 *Y. lipolytica* 已可实现 198.2 g/L 的琥珀酸产量[96]。这表明基于甲醇的琥珀酸体系仍需在产量与效率上实现进一步提升。

衣康酸在聚合物合成与光学材料制造中具有重要价值[97]。Severinsen 等[98]采用三阶段发酵策略，以甲醇和甘油为混合碳源，在 *K. phaffii* 中实现了 55.3 g/L 衣康酸的合成。尽管该产量仍低于土曲霉(*Aspergillus terreus*)在葡萄糖体系中达到的 150 g/L 水平[99]，但证实了甲醇作为衣康酸可持续生产替代碳源的可行性[98]，也为从甲醇合成其他 C5 有机酸提供了重要的研究基础。

4.2 脂肪酸和脂肪醇

微生物转化甲醇制备生物燃料是甲醇生物制造的重要方向，主要目标产物包括脂肪酸与

脂肪醇[33]。与糖类底物相比，甲醇具有更高还原力，与生物合成途径对还原当量的需求高度匹配[33]。在 *K. phaffii* 中，通过系统性重构中心碳代谢增强乙酰辅酶 A 与 NADPH 的供给，并阻断脂肪酸下游代谢途径以促进中间产物积累[81]。在补料分批发酵条件下，积累 23.4 g/L 脂肪酸与 2 g/L 脂肪醇，达到 *S. cerevisiae* 以葡萄糖为底物时的水平[81]。在 *O. polymorpha* 中，通过阻断脂肪酸下游代谢途径并结合 ALE 策略，恢复了菌株在甲醇中的生长能力，在补料分批发酵中实现 15.9 g/L 游离脂肪酸的积累[82]。Wang 等[39]在 *B. methylotrophicum* 中引入 rGly 途径，增强了甲醇和 CO_2 利用能力，进一步通过过表达外源 *adhE2* 基因，利用甲醇和二氧化碳合成了 1-丁醇，产量达到 1.4 g/L，是利用 C1 原料合成 1-丁醇的最高产量。

4.3 生物饲料

利用甲醇合成生物饲料可有效缓解饲料生产与粮食安全之间的竞争[84]。生物饲料包括饲料氨基酸和单细胞蛋白[33,84]。天然甲基营养菌可以甲醇为碳源合成氨基酸，如 L-谷氨酸、L-赖氨酸和 L-苏氨酸[33]。在改造 *K. phaffii* 中表达不同来源的 L-天冬氨酸脱羧酶(L-aspartate α-decarboxylase, ADC)，实现了以甲醇为原料合成 β-丙氨酸[83]。通过增强 ADC 表达强度、过表达天冬氨酸脱氢酶以提升前体天冬氨酸供给，进一步优化了合成途径，最终获得的工程菌株利用甲醇生产 β-丙氨酸产量达 5.6 g/L[83]。

甲基营养型酵母 *K. phaffii* 具备天然甲醇同化能力，是甲醇基 SCP 生产的重要底盘，研究者采用 ALE 策略提高了甲醇利用效率和温度耐受性，并进一步通过强化氮代谢和削弱细胞壁合成提高菌体蛋白含量[84]。最终，获得菌体干重 63.4 g/L，粗蛋白含量达 50.6%，甲醇转化率为 0.43 g DCW/g[84]。

4.4 生物医药产品

利用甲醇合成的生物医药产品主要有莫纳可林 J、洛伐他汀、硫酸软骨素、长春质碱等[85-87]。相比化学合成，生物合成反应条件温和、选择性高[85-87]。*K. phaffii* 通过异源组装莫纳可林 J 与洛伐他汀生物合成模块，并结合 pH 调控、双菌株共培养及生物反应器发酵，产量分别达到 0.594 g/L 和 0.251 g/L[85]。通过引入异源生物合成酶、优化启动子元件并整合硫酸化模块，在补料分批发酵中硫酸软骨素产量可达 2.1 g/L[86]。通过筛选稳定的基因组整合位点、挖掘高活性催化酶、重塑宿主代谢网络及优化发酵工艺，实现了长春质碱的从头合成[87]。以甲醇和甘露醇为碳源时，产量达到 2.57 mg/L[87]。在 *M. extorquens* AM1 中，通过构建与优化异源甲羟戊酸途径，α-葎草烯产量达 58 mg/L；结合补料分批培养与原位提取策略，其产量进一步提升至 1.7 g/L[88]。

3-HP、脂肪酸和 SCP 等与中心代谢紧密相关的产物已达到较好的产量，表明通过强化前体供应、辅因子再生和甲醇代谢调控可实现较高效的碳流转化[74,81,84]。然而，通常依赖复杂的中心代谢重构和精细发酵控制[74,81,84]。

复杂生物医药产品的甲醇生物合成目前整体仍处于低产量阶段。例如，莫纳可林 J、洛伐他汀和长春质碱等产物需要重构较长的天然产物合成途径，涉及多个异源酶、P450 酶、辅因子供给和中间体转运；通过途径拆分和共培养，莫纳可林 J 和洛伐他汀产量显著提高，表明共培养有助于缓解单一细胞中长途径表达带来的代谢负担[85]；但该策略也增加了菌株比例控制、体系稳定性和放大操作的复杂性。长春质碱的从头合成进一步证明了 *K. phaffii* 可承载超过 30 个异源基因的复杂植物天然产物途径，但产量仍处于毫克级，表明限速酶活性、途径平衡和宿主适配性仍是主要瓶颈[87]。

K. phaffii 是目前应用最广泛的甲醇生物制造底盘，具有遗传工具成熟、高密度发酵能力强、AOX1 启动子表达水平高等优势，因此在 3-HP、脂肪酸、β-丙氨酸、SCP、硫酸软骨素及

复杂天然产物合成中均有应用[51]。该底盘的甲醇代谢高度依赖过氧化物酶体和甲醇诱导调控网络，甲醛毒性、甲醇异化碳损失以及强表达带来的代谢负担仍是限制其进一步提高产量的关键因素。*O. polymorpha* 具有较好的甲醇利用能力和耐热性[8,77,79]，但工程化工具少于 *K. phaffii*。*S. cerevisiae* 和 *Y. lipolytica* 等非天然甲基营养酵母虽然具有成熟的工业应用基础和代谢工程体系，但需要重构甲醇同化途径[32]。*M. extorquens* 等天然甲基营养细菌适合萜类前体代谢，并已实现 α-葎草烯的较高产量[88]，但对于涉及 P450 酶、多酶复合途径或真核来源酶的复杂天然产物，甲基营养型酵母在表达真核来源酶以及构建多基因合成途径方面具有一定优势。

5 甲醇利用关键限速节点及工程改造

在甲醇生物转化过程中，甲醇氧化入口酶决定甲醇转化的初始通量，甲醛固定酶决定毒性中间体能否被快速承接，甲醛/甲酸氧化通路影响细胞耐受性及甲醇碳得率，而辅因子、电子传递和受体再生则进一步影响甲醇碳流向目标化学品的效率[33]。

甲醇氧化为甲醛是甲醇进入细胞代谢网络的起始步骤，相关功能酶主要包括甲醇氧化酶 MOX/AOX 和 MDH[22,24]。MOX/AOX 活性直接影响甲醇转化为甲醛的速率，活性过高会导致甲醛与 H_2O_2 积累，而活性过低则会限制甲醇碳流的输入[22]。Moser 等[100]通过适应性进化获得 4 个酶活降低的 AOX 突变体，对应的 *K. phaffii* 菌株在甲醇中的生长速率提升，重组蛋白在摇瓶和补料分批发酵中的产量分别提高 2.5 倍和 1.8 倍。与 AOX 相比，NAD^+依赖型 MDH 能够将甲醇氧化与 NADH 生成相耦联，但天然 MDH 存在催化活性低、底物特异性差以及与下游甲醛固定模块适配性不足等问题[101]。Roth 等[101]对 *Bacillus methanolicus* Mdh2 进行改造，获得最高催化速率提升 3.5 倍的突变体，使 ^{13}C-甲醇进入中心代谢的水平提高 2 倍。Keller 等[102]发现部分 NAD^+依赖型 MDH 会将甲醇进一步过度氧化为甲酸，造成不必要的碳损失；将过氧化型 Mdh2 替换为非过氧化型 MDH MGA3 后，胞内甲酸积累量降至 0.893 mmol/L，表明使用非过氧化型 MDH 是提升甲醇碳得率的重要策略。

甲醛固定酶是连接甲醇氧化和中心碳代谢的核心节点，其催化通量不仅取决于 Hps/Phi 或 Das/Dak，还受 Ru5P/Xu5P 受体再生、辅因子再生以及中心碳代谢通量分配的共同限制。Jiang 等[103]在 *Y. lipolytica* 中引入并强化 RuMP 模块，所得工程株在 24 h 内甲醇消耗量达到 2.35 g/L，生物量提高 2.68 倍，并可利用甲醇作为共底物合成白藜芦醇。在 *C. glutamicum* 中，异源表达 MDH-Hps/Phi 模块使工程菌甲醇消耗速率达到 (1.7±0.3) mmol/(L·h)[104]。在 *B. subtilis* 168 中，利用 SpyTag/Catcher 和 DogTag/Catcher 系统将 Mdh、Hps 和 Phi 组装后，并敲除 *fdhA*/*adhB*/*gnd* 及过表达 *glpX-tal-pgk*，工程菌株 SM6 可消耗 3.87 g/L 甲醇[57]。Price 等[105]将 NAD^+依赖型 Mdh3 与 Hps/Phi 组装成无支架多酶复合体，并引入 Ldh 促进 NAD^+再生，使体外 F6P 生成量较未组装酶体系提高 97 倍；在 *E. coli* Δ*frmA* 全细胞体系中，初始甲醇消耗速率由 0.19 mmol/(L·h) 提高至 1.7 mmol/(L·h)，约提高 9 倍。

XuMP 途径中的 Das/Dak 也可作为甲醛固定模块[26]。Wang 等[61]在 *Y. lipolytica* 中表达 *P. pastoris* 来源的 *Pp*DAS1/*Pp*DAK2，使甲醛积累量降低 28%；进一步构建包含 *Bs*MDH、*Bm*HPS/*Bm*PHI 和 *Pp*DAS1/*Pp*DAK2 的嵌合 RuMP/XuMP 通路后，工程菌 72 h 甲醇消耗量达到 0.42 g/L，在进一步强化 Ru5P/Xu5P 再生模块后，甲醇消耗量较初始嵌合通路菌株提升 43%。

甲醛/甲酸氧化在甲醇利用中有双重作用。FLD、FGH、FDH、Fae 及部分底盘中的 Ald/

AdhE 等甲醛活化、甲醛氧化或甲酸氧化相关酶可以降低甲醛毒性、维持细胞生长，但过强的氧化通量会将甲醇来源的碳进一步转化为 CO_2，导致碳得率低[33]。Jiang 等[103]研究发现 *Y. lipolytica* 中过表达 MDH 构建 Po1dM，48 h 可消耗 0.6 g/L 甲醇；但删除 *fld* 后，Po1dM-fldKO 的甲醇消耗能力降至野生型水平。Witthoff 等[104]研究发现，*C. glutamicum* 敲除 *ald* 和 *adhE* 以削弱甲醛氧化为 CO_2，甲醇来源的碳进入胞内代谢物的比例提高约 2.5−3.0 倍。

甲醇碳流进入目标产物合成途径后，辅助循环或产物前体供应模块会限制甲醇的转化效率。Li 等[106]发现在 *K. phaffii* 中存在参与甲醇碳流转化的内源同型丝氨酸循环，其中丝氨酸脱水酶(serine dehydratase, SDA)和苏氨酸合成酶(threonine synthase, TS)是该循环的关键限速酶；进一步优化其拷贝数，发现三拷贝 TS 的工程菌株，其 β-胡萝卜素产量达到 173.9 mg/L，单位细胞产物产量较初始菌株提升 145.7%。

因此，甲醇的高效利用需要综合协调甲醇氧化、甲醛固定、受体再生、中心代谢承接和产物合成各模块之间的通量平衡。未来研究应进一步结合适应性实验室进化、多组学分析、动态调控、多酶组装、区室化工程和机器学习辅助酶工程等策略，构建可在高甲醇通量、低毒性积累和高碳得率三者间实现动态平衡的甲醇生物转化体系。

6 甲醇生物制造技术经济分析

甲醇作为液态 C1 原料具有储运方便、易于和传统发酵设备兼容等优势，但其经济性高度依赖原料来源[3]。化石甲醇成本相对较低，约 200−400 EUR/t，低于葡萄糖或蔗糖等糖基原料[107]。电甲醇(e-methanol)目前仍受可再生电力、绿氢和 CO_2 捕集成本限制，生产成本较高，2020 年成本约为 1 200−1 500 EUR/t [107]。然而，根据预测，电甲醇的价格会逐步降低，2030 年约 600−680 EUR/t、2040 年约 390−430 EUR/t 和 2050 年约 315−350 EUR/t[107]。

除甲醇原料价格外，当前甲醇基生物制造的技术成本主要来自甲醇和甲醛毒性控制[108]、甲醛生成与同化通量平衡[15,33,108]、辅因子和前体再生[69]、好氧发酵供氧与冷却需求[33,109]、菌株构建与适应性进化成本[33]，以及低滴度、低产率和低生产强度导致的设备占用和下游分离成本[109-111]。技术经济分析(techno-economic analysis, TEA)研究表明，在 C1 生物级联和电-生物级联路线中，C1 原料到化学品的总体碳转化效率低于 10%，低碳收率会增加原料消耗和反应器规模，从而同时推高运营支出(operating expenditure, OPEX)和固定资产投资(capital expenditure, CAPEX)[111]。因此，提高甲醇碳向目标产物的转化效率、减少甲醛异化和副产物生成、提高产物滴度与生产强度，是降低甲醇基生物制造成本的关键[111]。

KnipBio 公司利用甲基营养菌 *M. extorquens* 以天然气甲醇作为碳源，经工业发酵生产单细胞蛋白，用作水产动物饲料蛋白原料[112]。该产品已经获得美国食品药品监督管理局(Food and Drug Administration, FDA)的相关认可[112]。

7 总结与展望

甲醇作为 C1 底物具有来源广、还原度高和液态易输送等优势，但其实际应用仍受到理化安全性和生物转化效率的双重限制(表 4)。甲醇易挥发、易燃，具有很大的安全隐患[113-114]。此外，甲醇进入细胞后先氧化为甲醛，随后进入同化或异化途径，该过程容易引发甲醛/甲酸积累、H_2O_2/ROS 氧化胁迫、碳流向 CO_2 引起碳损失以及 NADH/NAD^+失衡等问题[108]。以上问题的解决方案包括过程控制及代谢工程[33,113]。过程控制包括密闭输送、在线补料控制、共底物补料和尾气生物过滤[113-115]。代谢工程包括甲醇同化途径重构、MDH/AOX 工程、甲醛受体再生、抗氧化系统强化[122]、膜脂工程[119]、区室化[120-121]和适应性进化等[124]。

表4 甲醇利用存在的难点和解决策略

Table 4 Key challenges and corresponding strategies for methanol utilization

Key challenge	Corresponding strategies	References
Methanol vapor emissions in fermentation off-gas	Treat methanol-containing off-gas by biofiltration, biotrickling filtration, or biofilter-photobioreactor coupling	[113-114]
Narrow methanol feeding window and difficulties in process control	Implement feedback-controlled feeding based on online methanol monitoring, DO-stat, or ON-OFF control; apply limiting or intermittent feeding strategies; reduce peak methanol concentrations through co-substrate feeding, such as methanol/sorbitol or methanol/glycerol feeding	[115-117]
Oxygen transfer and heat dissipation limitations during high-cell-density fermentation with methanol	Optimize aeration, agitation, and cooling systems; reduce the instantaneous methanol feeding load; use co-substrate feeding to share the energy supply burden; optimize AOX/MDH oxidation rates to alleviate excessive oxygen demand, heat generation, and oxidative stress	[115, 117-118]
Toxicity of methanol oxidation products and oxidative stress	Enhance formaldehyde detoxification, dissimilation, and assimilation; improve the regeneration of formaldehyde acceptors such as Xu5P and Ru5P; strengthen antioxidant systems involving CAT and GSH; reduce formaldehyde leakage/toxicity through membrane lipid engineering and compartmentalization	[116, 119-121]
Low methanol assimilation efficiency, carbon loss, and insufficient chassis adaptability	Reconstruct methanol assimilation pathways, including the RuMP pathway, XuMP pathway, and reductive glycine pathway; optimize MDH/AOX activity and cofactor balance; minimize carbon loss through dissimilation; select natural methylotrophs or engineer chassis strains through ALE, omics-guided engineering, and membrane lipid engineering	[122-125]

甲醇生物转化虽然在天然和人工甲醇同化途径解析、甲基营养菌构建及高附加值化学品的合成方面取得一定进展，但是实际应用仍面临很多挑战，例如甲醇及其氧化中间体毒性高、与下游同化通量不匹配、甲醛同化模块效率有限及异源途径和宿主适配性差、甲醇碳流进入中心代谢和目标产物合成效率有限等关键挑战。未来研究应进一步围绕甲醇氧化、甲醛固定、碳一受体再生、辅因子平衡和产物合成通量之间的协调展开；结合适应性实验室进化、反向工程和多组学技术分析，挖掘甲醇耐受、高效同化等的关键靶点，解析甲醇代谢调控机制。进一步构建甲醇利用速率高、碳转化率高的微生物底盘，持续推动甲醇高效生物转化。

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作者利益冲突公开声明

作者声明不存在任何可能会影响本文所报告工作的已知经济利益或个人关系。

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