特约评述

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微生物合成高级醇的发展趋势与挑战

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摘要：高级醇是指含有三个或以上碳原子的醇类。传统高级醇的生产主要依赖于石化资源，然而其不可再生性限制了相关产业的发展，因此开发可持续的生物基高级醇生产技术成为研究热点。本文综述了高级醇的市场规模、主要应用领域及其经济价值，并重点分析了异丁醇、1,3-丁二醇和2,3-丁二醇的市场表现。进一步探讨了高级醇的生物合成路径，包括乙酰辅酶A依赖途径、支链氨基酸合成途径和脂肪酸链延长途径，同时总结了代谢工程优化策略，如辅因子平衡调节、竞争途径敲除、酶优化及高产菌株筛选。此外，本文还综述了基于新技术的多维度优化策略，未来有望通过生物传感器、高效基因编辑和计算机辅助代谢工程等技术的结合，进一步优化微生物细胞工厂的设计，有助于提高高级醇的工业化生产效率，为可再生能源和绿色化学工业的发展提供重要支持。

关键词：生物燃料；高级醇；合成途径；代谢工程；合成生物学

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Trends and challenges in microbial synthesis of higher alcohols

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Abstract: Higher alcohols refer to alcohols containing three or more carbon atoms and represent an important class of chemicals widely used in various industries, such as fuels, solvents, coatings, and specialty chemicals. Traditionally, the production of these higher alcohols has depended heavily on petrochemical processes, which not only rely on non-renewable resources but also contribute significantly to environmental pollution. The finite nature of fossil fuels and the associated environmental concerns have prompted researchers to explore alternative, sustainable production

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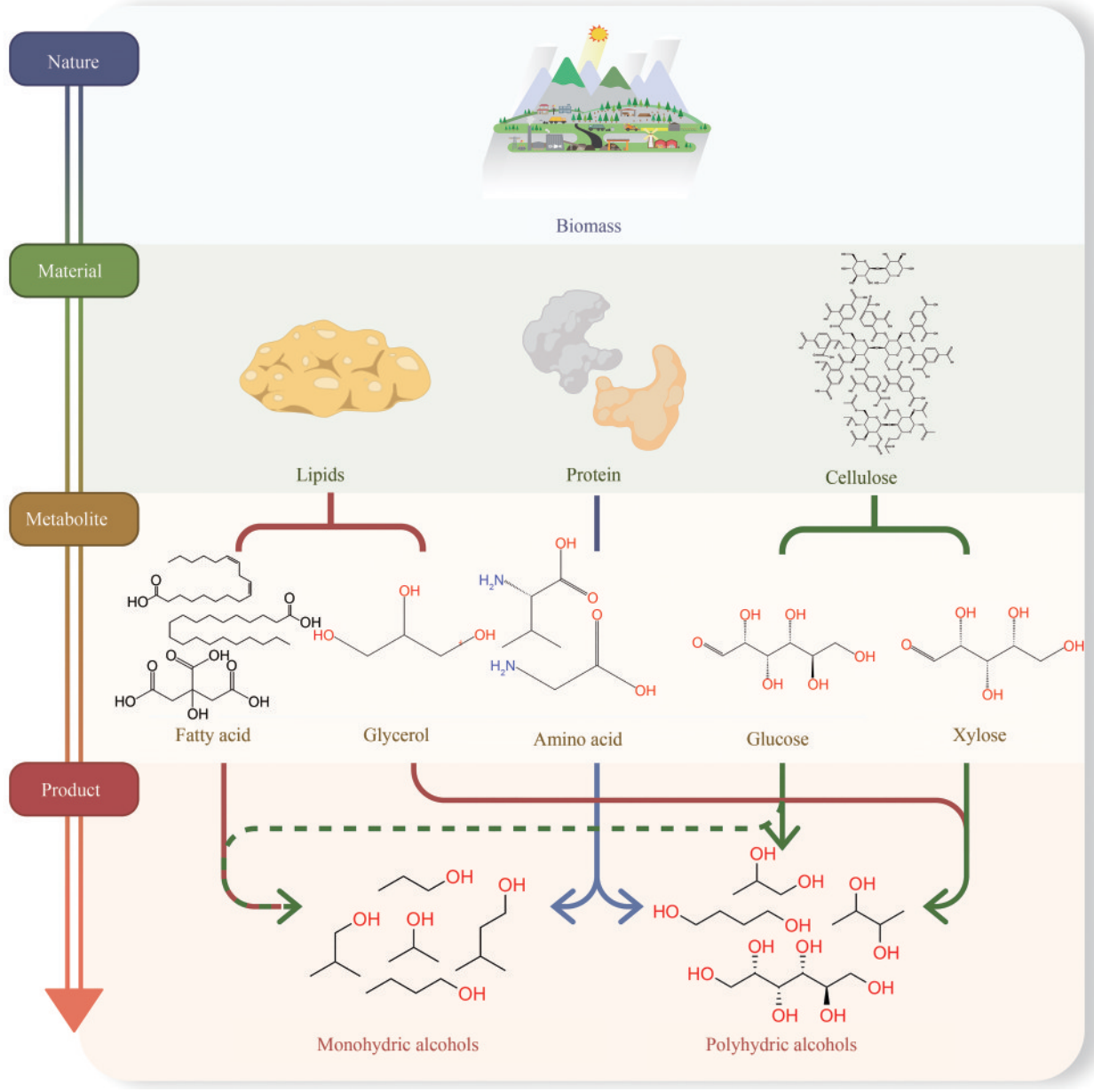
methods. Consequently, the development of sustainable bio-based higher alcohol production technologies has emerged as a critical research focus. Recent advances in metabolic engineering and synthetic biology have paved the way for developing engineered microbial strains capable of producing higher alcohols through biological fermentation. By optimizing metabolic pathways, these engineered strains can channel more carbon flux toward the desired alcohol products. In addition, enhancing the tolerance of these strains to high concentrations of the produced alcohols and improving their overall biosynthetic capabilities are key strategies that have been successfully implemented. Such innovations have enabled the production of higher alcohols from renewable feedstocks in a more environmentally friendly and cost-effective manner. Renewable raw materials, such as lignocellulose, waste proteins, waste lipids, and carbon dioxide, provide diverse possibilities for the environmentally friendly and sustainable synthesis of higher alcohols. Lignocellulosic biomass, for instance, is abundant and renewable, making it an attractive alternative to conventional sugars. Waste proteins and lipids, often derived from industrial by-products, provide additional inexpensive substrates that not only help in waste valorization but also reduce the overall production cost. Carbon



dioxide, as an abundant greenhouse gas, can be captured and converted into valuable higher alcohols, contributing to carbon sequestration and climate change mitigation. Despite these promising prospects, several challenges remain to be addressed. Issues such as low substrate conversion efficiency, the formation of inhibitory byproducts during fermentation, and high costs associated with downstream separation and purification continue to hinder commercial viability. This review provides a comprehensive overview of the current market size, major applications, and economic value of higher alcohols, with particular emphasis on the market performance of isobutanol, 1, 3-butanediol, and 2, 3-butanediol. In addition, the review explores the biosynthetic pathways utilized for higher alcohol production, including the acetyl-CoA-dependent pathway, the branched-chain amino acid synthesis pathway, and the fatty acid chain elongation pathway. It also summarizes key metabolic engineering strategies, such as cofactor balancing, competitive pathway elimination, enzyme optimization, and high-yield strain selection. Moreover, the utilization of extremophiles as chassis cells, in combination with next-generation industrial biotechnology (NGIB), represents a promising new direction for sustainable production. Looking ahead, the integration of biosensors, advanced gene editing technologies, and computer-aided metabolic engineering is expected to further optimize microbial cell factory design, thereby enhancing the industrial production efficiency of higher alcohols and promoting the development of renewable energy and green chemical industries.

Keywords: biofuels; higher alcohols; synthetic pathway; metabolic engineering; synthetic biology

根据高级醇羟基数目的不同，可将其分为两类：一元醇与多元醇。其中，一元醇包括正丙醇（*n*-propanol，*n*-PrOH）、异丙醇（isopropanol，IPA）、异丁醇（isobutanol，IBOH）、正丁醇（*n*-butanol，*n*-BuOH）、2-丁醇（2-butanol，2-BuOH）、2-甲基-1-丁醇（2-methyl-1-butanol，2M1B）、3-甲基-1-丁醇（3-methyl-1-butanol，3M1B）等；多元醇包括1，2-丙二醇（1，2-propanediol，1，2-PDO）、1，3-丙二醇（1，3-propanediol，1，3-PDO）、1，3-丁二醇（1，3-butanediol，1，3-BDO）、2，3-丁二醇（2，3-butanediol，2，3-BDO）、1，4-丁二醇（1，4-butanediol，1，4-BDO）、1，2，4-丁三醇（1，2，4-butanetriol，BT）、木糖醇（xylitol）、赤藓醇（erythritol）和甘露醇（mannitol）等。在化工领域，多元醇是聚氨酯弹性体与多种聚合物的关键原料，广泛应用于基础设施、鞋类制造、汽车工业及机械设备[1-2]；在能源领域，一元醇可作为燃料添加剂部分替代传统化石燃料，能够降低碳排放强度[3]。此外，高级醇的应用范围还涉及防冻剂[4]、表面活性剂[5]及化妆品[6]等细分市场。

传统高级醇的工业化生产高度依赖石油资源，然而石油的不可再生性严重制约了行业可持续发展。为减少对石油资源的依赖，近年来生物制造技术成为研究热点。通过代谢工程策略重构工业菌株的合成途径，显著提升了目标产物的积累效率与菌株稳定性。同时，原料来源的多元化探索为降低生产成本提供了新方向。尽管如此，现有生物制造体系仍面临多重挑战，包括微生物代谢产物浓度低、下游分离纯化能耗高、生物质转化效率不足，以及菌株对抑制剂的耐受性有限。此外，高温高压反应条件与催化剂的使用进一步增加了工艺复杂性与操作成本。将高级醇合成的原料扩展为低成本的纤维素、蛋白质及二氧化碳（CO_2），有望提高生产经济性[7-8]。相较于传统生物制造，基于极端微生物的下一代工业生物技术（next generation industrial biotechnology， NGIB）展现出独特优势。NGIB通过利用极端微生物对高盐、高温等逆境的天然耐受性，可在开放式发酵体系中创造选择性生长环境，从而抑制杂菌增殖并避免污染[9-11]。本文系统综述了高级醇的市场动态及生物制造技术的最新进展，重点解析了代谢工程策略的创新逻辑与技术瓶颈，并展望了智能化合成生物学平台对产业未来的变革性影响。同时本文通过整合多学科交叉研究成果，旨在为高级醇的工业化生产与可持续应用提供理论依据及可落地的技术路线参考，拓展其在可降解塑料及高附加值精细化学品等领域的应用，从而为构建全球可再生能源体系和完善循环经济模式提供关键技术支撑。

1 高级醇的市场规模与经济效益

高级醇主要应用于化学工业（35%）、生物燃料（25%）、食品和饮料（15%）、制药行业（10%）、化妆品（10%）以及其他工业（5%），随着消费者对天然成分需求增长以及绿色消费趋势推动，高级醇在食品与化妆品领域的市场占比预计将进一步提升[12-13]。全球多元醇市场正处于稳健增长周期，市场规模达295.1亿美元，预计2032年扩张至413.7亿美元[14]。多元醇凭借羟基官能团的化学多样性及低成本制造特性，可适配聚氨酯、涂料、胶黏剂等广泛下游应用场景。其中，建筑领域贡献显著增量，全球节能建筑政策[15]推动聚氨酯硬质泡沫需求激增，该材料作为高效隔热体可降低建筑能耗30%～50%，并同步减少温室气体排放。据测算，2024年建筑领域占多元醇消费量的比重已超45%。与此同时，生物基多元醇因其低碳属性，在碳中和目标下渗透率加速提升，进一步强化行业增长动能[14]。

异丁醇作为重要的化学中间体，广泛应用于合成溶剂、涂料、塑料添加剂、风味剂和香料等行业中[16-17]。2025年异丁醇价格因地区和纯度差异较大，国产优等品价格区间为7400～10500元/吨。其中山东地区异丁醇（含量99.9%）价格区间为7400～8000元/吨，江苏地区异丁醇（含量99.9%）价格区间为8500～10500元/吨[18]。根据行业监测数据，截至2024年12月，全球异丁醇市场呈现显著的区域分化特征，北美地区因环保政策收紧与化工产业链调整，市场同比萎缩31%；欧洲市场受能源成本高企及终端需求疲软影响，同比下降12%。在全球异丁醇市场分化的背景下，亚太地区尽管面临原料供应波动推高生产成本，但在涂料行业需求复苏的支撑下，异丁醇价格同比上涨6%[19]。

1,3-丁二醇主要在化妆品行业作为保湿剂被广泛应用，在食品、饮料及化妆品行业中均占比约15%。在化学工业中作为化学中间体和溶剂，用于生产增塑剂、聚合物和涂料占化学工业应用约40%。在制药行业作为药物中间体和溶剂，占比约为20%。此外，随着绿色能源转型加速，1,3-丁二醇在生物燃料领域占比约10%，成为新兴增长点。根据QYResearch最新调研报告显示[20]，2024年全球1,3-丁二醇市场规模为2.29亿美元，预计2031年将达到3.74亿美元，主要驱动力来自化妆品与医药中间体需求的扩张，而传统溶剂市场增长趋缓。中国作为核心消费国仍面临结构性失衡，2025年医药级1,3-丁二醇进口依赖度超80%[21]，主要受限于菌种专利壁垒与生物发酵技术短板。当前产业正经历从细分应用向规模化生产的转型，其突破需依托合成生物技术创新与产业链政策协同，以实现技术自主性与市场竞争力提升。

2,3-丁二醇是一种重要的化学中间体，广泛应用于工业生产中，包括合成塑料、溶剂、电子化学品和精细化学品等领域[16, 22-23]。当前，2,3-丁二醇的市场需求呈现持续增长态势，其核心驱动因素源自制药工业的快速扩张——作为合成手性药物中间体及生物活性分子的关键原料，在创新药物研发中具有不可替代性。据Market Research Future预测，2024年全球2,3-丁二醇市场规模预计达13.5亿美元，并将在2025—2034年以9.16%的年均复合增长率（compound annual growth rate，CAGR）持续扩张，至2034年市场规模将突破32.5亿美元[24]。

高级醇的产业化发展受技术创新、政策支持与市场机制优化的三重驱动，核心逻辑在于生物基化学品需求的增长与绿色化工转型的战略协同。技术创新聚焦于微生物代谢工程与酶催化效率提升，政策层面则体现为财政激励（如生物基产品补贴、碳税减免）及产业扶持，而市场机制需通过供需结构优化与产业链协同降低交易成本。然而，政策周期波动与标准体系不完善可能引发投资风险，如欧盟生物基产品认证的严苛性制约技术转化效率。因此，实现高级醇作为可再生能源载体的潜力，需构建“技术-政策-市场”三位一体的协同发展框架，通过菌种专利池建设、绿色金融工具创新及碳交易市场联动，系统性降低全生命周期成本，从而为能源结构优化与碳中和目标提供可扩展的产业支撑（图1）。

2 高级醇的原料来源

在全球地缘政治复杂性加剧与能源安全挑战

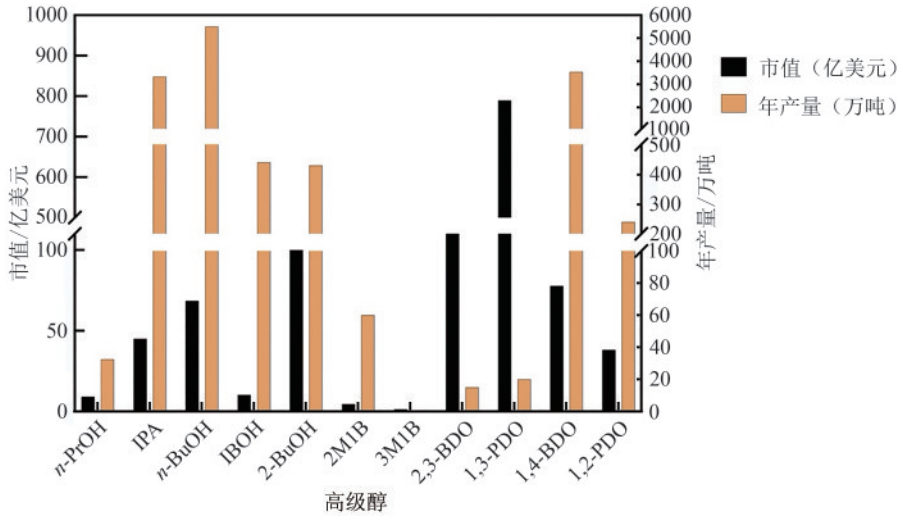


图1 高级醇年产量与市值

Fig. 1 Annual production and market value of higher alcohols

凸显的背景下，中国于2020年联合国大会明确提出“双碳”目标（2030年碳达峰、2060年碳中和），这一战略导向加速了生物基材料对石化产品的替代进程[25]。木质纤维素、废弃蛋白质、废弃油脂及CO_2等生物基材料因具有碳封存潜力与可再生属性，成为实现碳减排的关键介质，然而其产业化面临多重技术壁垒。预处理能耗占木质纤维素总生产成本的35%以上，而微生物对混合碳源的代谢效率不足50%；CO_2催化转化虽具负碳效应，但依赖高温高压条件及贵金属催化剂，导致能量转化效率低于30%。因此，开发低能耗预处理技术、构建多碳同化模块化细胞工厂，将成为突破生物制造技术经济性瓶颈、保障能源体系低碳转型的核心攻关方向。

2.1 木质纤维素

木质纤维素作为地球上最丰富的可再生生物质资源之一，来源广泛、成本低廉且具有碳中和属性，年产量达181.5亿吨，使木质纤维素成为生物基化学品生产的理想原料[26-28]。目前，以木质纤维素为原料已开发出200余种增值化学品[29]。在高级醇合成领域，木质纤维素需经过预处理转化为葡萄糖和木糖，才能被微生物有效利用（图2）。有研究表明，通过优化纤维素酶解效率，技术改进使葡萄糖产量提升了674%[30]。工业酵母菌株MDS130在未脱毒的木质纤维素水解液中同步代谢葡萄糖与木糖，乙醇收率达理论值的91%[31]。针对木质素的高效利用，聚乙二醇（polyethylene glycol，PEG）修饰的加氢降解法可显著提高木质素的溶解度和单木酚产率，但去除PEG后效率下降[32]。此外，研究通过调整pH值优化木糖和木质素的分离纯化，以及两步温和降解工艺的应用，玉米芯木糖产率可提升至97%[33-34]。

然而，木质纤维素的结构复杂性仍严重制约其规模化应用：其一，纤维素、半纤维素与木质素的致密交联结构导致微生物直接利用困难[27]；其二，木质素降解过程存在效率低、催化剂失活及产物选择性差等技术瓶颈；其三，预处理生成的呋喃类、酚类抑制物会显著抑制微生物生长并降低发酵效率。当前的核心挑战在于开发高效酶解体系、抗逆性菌株及低毒性预处理技术。未来需通过催化剂理性设计、酶工程化改造及生物降解路径优化，突破技术经济性瓶颈，从而充分发挥木质纤维素资源丰富性、可再生性及碳减排优势，推动生物制造产业向可持续发展方向升级。

2.2 油脂

以油料、废弃食用油及生物柴油副产物甘油为原料生产高级醇，兼具资源利用率高与环境友好性强的双重优势。2023年，我国油料总产量达3864万吨，同比增长5.7%[35]，其中大豆因油酸含量高达21.95%[36]，有望成为生物柴油及高级醇合

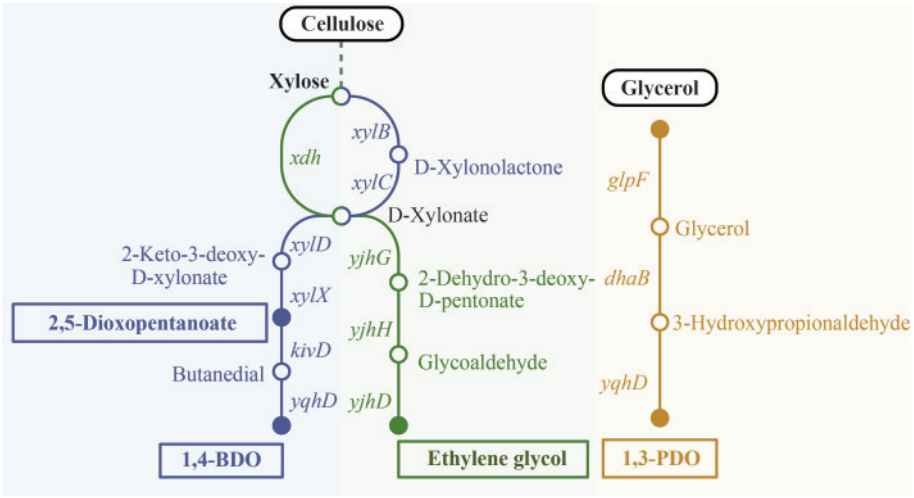


图2 纤维素代谢合成高级醇的途径

（1,3-PDO—1,3-丙二醇；1,4-BDO—1,4-丁二醇；*glpF*—甘油通道蛋白编码基因；*dhaB*—甘油脱氢酶编码基因；*kivD*—α-酮异戊酸脱羧酶编码基因；*yqhD*—NADPH依赖的醛还原酶YqhD编码基因；*xylB*—木酮糖激酶编码基因；*xylC*—木糖内酯酶XylC编码基因；*xdh*—黄嘌呤脱氢酶编码基因；*yjhH*—2-脱氢-3-脱氧-D-戊酮酸醛缩酶编码基因；*yjhG*—D-木糖酸脱水酶编码基因；*xylX*—甲苯甲酸1,2-双加氧酶α亚基编码基因）

Fig. 2 Metabolic pathway of higher alcohol synthesis from cellulose

(1,3-PDO—1,3-Propanediol; 1,4-BDO—1,4-Butanediol; *glpF*—glycerol facilitator gene; *dhaB*—glycerol dehydratase gene; *kivD*—alpha-ketoisovalerate decarboxylase gene; *yqhD*—NADPH-dependent aldehyde reductase YqhD gene; *xylB*—xylulokinase gene; *xylC*—xylonolactonase XylC gene; *xdh*—xanthine dehydrogenase gene; *yjhH*—putative 2-dehydro-3-deoxy-D-pentonate aldolase gene; *yjhG*—D-xylonate dehydratase gene; *xylX*—toluate 1,2-dioxygenase subunit alpha gene)

成的优选原料；废弃食用油作为重要脂肪酸来源，其回收利用既可减少环境负担，又能实现资源增值[37]；而生物柴油副产物甘油通过生物催化技术可高效转化为附加值产物[38]。在微生物代谢层面，油脂原料的优势体现于其天然脂肪酸代谢网络的可编辑性[39]；此外，甘油因其水溶性及强还原性，更易被微生物同化并驱动还原性产物的生物合成。针对废弃油脂的高水分与杂质问题，目前已建立脱酸、脱水等预处理技术体系[40]；通过重构内源性脂肪酸合成途径，脂肪醇的生物合成效率得到显著提升[39]（图2）；在甘油转化领域，基于代谢网络的动态调控，1,3-丙二醇与3-羟基丙酸（3-HP）的协同合成已实现突破[38]。

目前，基于油料与废弃油脂的高级醇生产已迈入中试阶段，例如甘油衍生1,3-丙二醇的工业化产能突破万吨级[38]；微生物合成脂肪醇的实验室产量达克级水平[39]。然而，该领域仍面临严峻挑战：其一，废弃油脂成分复杂且物化性质不稳定，导致微生物底物利用效率低下及产物纯度不足；其二，脂肪酸直接转化为高级醇的研究体系尚未完善，现有技术依赖多步间接路径，存在催化选择性低与能量损耗高等问题；其三，微生物利用油脂时易受底物毒性抑制，且需精准平衡脂肪酸β-氧化与目标产物合成的代谢通量。此外，预处理副产物及甘油转化过程中3-羟基丙酸的积累对菌体生长的抑制[38-40]，进一步增加了工艺调控难度。因此，未来需聚焦于高活性、耐逆型油脂降解酶元件的创制，亟待通过代谢通量精准调控与抗毒性强化策略构建高效工程菌株，同时结合低能耗预处理与产物原位分离工艺的系统性优化，深度释放油脂原料碳密度高、可再生性强及成本低廉的优势，加速生物制造技术向绿色低碳范式转型升级。

2.3 蛋白质

蛋白质是生物质精炼过程中发酵残渣的主要成分之一，其碳、氮元素的可循环特性为生物基化学品生产提供了潜在的原料基础[41]。将废弃蛋白质转化为高级醇，不仅可实现资源再生利用，而且可以缓解氮污染问题，契合可持续化学与生物燃料领域的低碳化需求。微生物利用蛋白质的核心优势在于其天然蛋白酶系的高效降解能力，通过分泌蛋白酶将蛋白质水解为氨基酸，既可将

其作为氮源参与细胞代谢，又能通过脱氨、脱羧等反应生成酮酸中间体，进而转化为异丁醇、异戊醇（isopentanol）等高级醇[42-43]（图 3）。此外，借助基因工程强化蛋白酶表达或优化氨基酸代谢节点，可定向调控碳流分配以提升目标产物合成效率[43]。自 1976 年发现氨基酸可作为氮源参与醇类代谢以来，相关研究持续深化[42]。近年来，通过基因编辑技术强化酿酒酵母（*Saccharomyces cerevisiae*）、梭菌（*Clostridium*）等微生物的氨基酸代谢通路，异丁醇等产物的合成效率显著提升[43]。当前研究聚焦于酶工程与代谢路径解析：一方面，利用生物信息学工具预测蛋白质功能并解析催化机制，结合定向进化技术提升酶的热稳定性与催化活性[44-45]；另一方面，通过分析蛋白质降解产物的挥发性化合物谱，揭示向特定化学产物的转化规律，为技术开发提供理论支撑[46]。

目前，微生物转化蛋白质合成高级醇的研究尚未规模化，异丁醇等产物的理论得率不足 0.3 g/g[43]，距产业化应用尚有较大差距。原料方面，从复杂废弃物中高效提取高纯度蛋白质面临能耗高、原料异质性显著等技术难题，导致蛋白质水解效率稳定性不足；在微生物代谢过程中，工业菌株普遍存在胞外蛋白酶分泌能力低下、氮代谢失衡引发的毒性抑制以及碳氮代谢流协同调控困难等问题，需通过代谢工程精准平衡氨基酸分解与酮酸转化的动态通量；技术层面，目前微生物转化蛋白质合成高级醇的研究仍处于实验室探索阶段，且产物分离成本居高不下。未来需重点突破高效、低成本蛋白质提取工艺，蛋白酶高表达工程菌株构建及碳氮代谢动态调控系统设计，以充分发挥

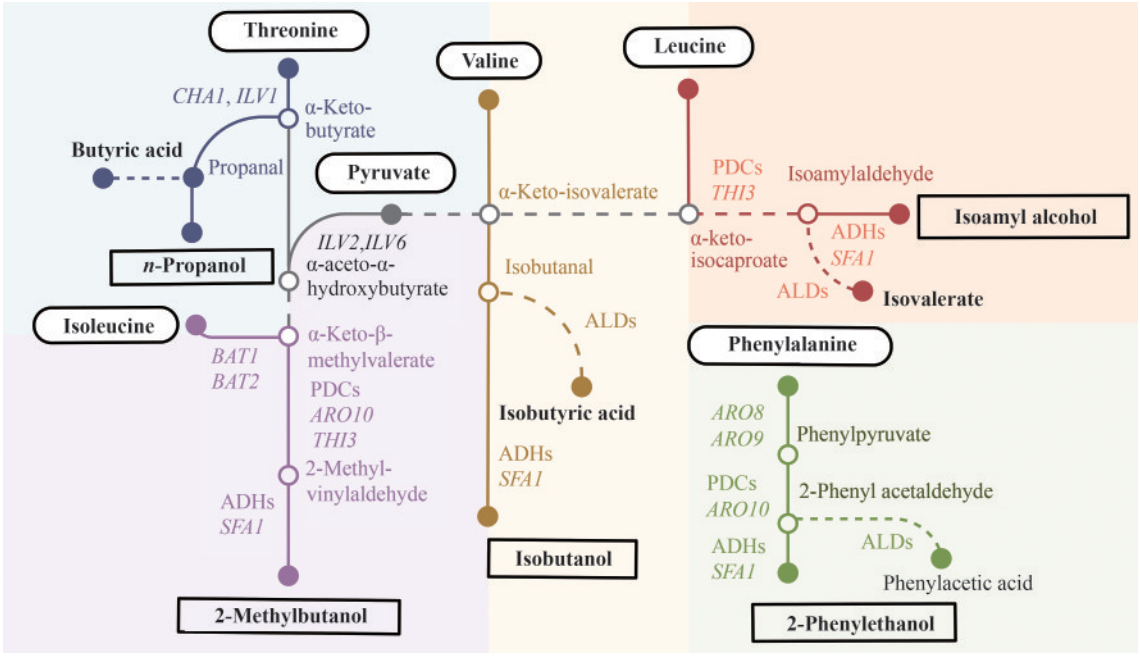


图 3　基于蛋白质代谢的高级醇生物合成途径

（*CHA1*—L-丝氨酸/L-苏氨酸氨裂解酶 CHA1 编码基因；*ILV1*—苏氨酸氨裂解酶 ILV1 编码基因；*BAT1*—支链氨基酸转氨酶 BAT1 编码基因；*BAT2*—支链氨基酸转氨酶 BAT2 编码基因；PDCs—丙酮酸脱羧酶；*THI3*—支链-2-氧酸脱羧酶 THI3 编码基因；ADHs—醇脱氢酶；*SFA1*—双功能乙醇脱氢酶/*S*-（羟甲基）编码基因；*ARO10*—苯基丙酮酸脱羧酶 ARO10 编码基因；*ARO8*—双功能 2-氨基己二酸转氨酶/芳香族氨基酸：2-酮戊二酸转氨酶编码基因；*ARO9*—芳香族氨基酸：2-氧代戊二酸转氨酶编码基因；ALDs—醛脱氢酶；*ILV2*—乙酰乳酸合成酶催化亚基编码基因；*ILV6*—乙酰乳酸合成酶调节亚基编码基因）

Fig. 3　Biosynthetic pathways of higher alcohols based on protein metabolism

(*CHA1*—L-serine/L-threonine ammonia-lyase CHA1 gene; *ILV1*—threonine ammonia-lyase ILV1 gene; *BAT1*—branched-chain-amino-acid transaminase BAT1 gene; *BAT2*—branched-chain-amino-acid transaminase BAT2 gene; PDCs—pyruvate decarboxylases; *THI3*—branched-chain-2-oxoacid decarboxylase THI3 gene; ADHs—alcohol dehydrogenases; *SFA1*—bifunctional alcohol dehydrogenase/*S*-(hydroxymethyl) glutathione dehydrogenase gene; *ARO10*—phenylpyruvate decarboxylase ARO10 gene; *ARO8*—bifunctional 2-aminoadipate transaminase/aromatic-amino-acid: 2-oxoglutarate transaminase gene; *ARO9*—aromatic-amino-acid: 2-oxoglutarate transaminase gene; ALDs—aldehyde dehydrogenases; *ILV2*—acetolactate synthase catalytic subunit gene; *ILV6*—acelolactate synthase regulatory subunit gene)

蛋白质原料碳氮双循环、环境兼容性强的核心优势，推动生物精炼技术向闭环资源化方向升级。

2.4 二氧化碳

CO_2作为全球碳循环的核心组分广泛存在于大气中，浓度约为424 μL/L[47]。2023年CO_2排放量达到374亿吨，较前一年增长1.1%[48]。目前CO_2主要用于生产尿素生产、油气开采及甲醇等工业化学品合成，年消耗量分别为1.3亿吨和7000万至8000万吨[49-53]，但作为可再生原料合成高附加值产物如高级醇的潜力仍待深入开发。CO_2的转化优势源于其来源广泛性和碳中和属性，工业排放与大气中的CO_2可无限供给，突破传统原料的供应链限制；结合生物固碳技术，可同步实现碳减排与碳中和战略目标。自养微生物如*Cupriavidus necator*通过卡尔文循环等天然代谢途径同化CO_2的特性进一步凸显其代谢兼容性。近年来微生物固碳技术取得突破，例如基因编辑技术重构*Cupriavidus necator* H16的代谢网络，定向改造其无机碳代谢路径，为CO_2转化为高级醇提供新策略[54]。此外嗜酸氧化亚铁硫杆菌（*Acidithiobacillus ferrooxidans*）的差异化固碳效率研究为工艺优化提供依据[55]，而大气CO_2与O_2水平对氮同位素分馏的影响解析则揭示了环境因素与代谢稳定性的关联机制[56]。

当前CO_2生物转化技术仍处于实验室阶段，其效率远逊于传统糖基工艺。在CO_2生物转化高级醇过程中，多碳醇合成需经历C_1至C_{3+}的碳链延伸过程，但关键酶活性不足会导致转化效率低下。CO_2还原所需的大量ATP与还原力受限于自养微生物的低能量代谢效率。与此同时高浓度CO_2引发的细胞酸化与氧化应激、高级醇积累导致的膜损伤以及外源抑制剂如咪唑类化合物的酶活性干扰共同构成毒性抑制网络，加之长期连续发酵中代谢网络的动态失稳问题亟待解决[57-58]。未来需构建高活性CO_2固定酶复合体以提升碳链延伸效率，设计光能或电能驱动的能量供应模块破解代谢瓶颈，并开发抗逆菌株集成原位分离工艺以降低毒性效应。通过多维度创新有望将转化效率提升至工业化阈值，从而发挥CO_2可再生性、低成本及环境兼容性优势，推动碳负制造技术的规模化应用[49-51]。

3 高级醇的生物合成

在微生物代谢工程领域，高级醇的生物合成体系已形成以天然代谢网络优化与人工途径创新为核心的双轨策略。在高级醇中一元醇的合成主要通过两条代谢途径实现。氨基酸衍生途径与乙酰辅酶A（acetyl-CoA）依赖途径（图4）。其中，乙酰辅酶A依赖途径是1-丁醇、2-丁醇及异丁醇等短链丁醇异构体的核心生物合成途径。在CoA依赖途径中，葡萄糖首先通过糖酵解途径（Embden-Meyerhof-Parnas pathway， EMP）生成丙酮酸，丙酮酸通过丙酮酸-铁氧还蛋白氧化还原酶催化脱羧，转化为乙酰辅酶A；2分子乙酰辅酶A在硫解酶（thiolase）作用下缩合为乙酰乙酰-CoA，随后经β-羟基丁酰-CoA脱氢酶（Hbd）及丁酰-CoA脱氢酶（Crt）催化，逐步形成丁酰-CoA；丁酰-CoA通过丁醛脱氢酶（Bdh）与丁醇脱氢酶（AdhE）的级联还原反应生成丁醇类化合物[59]。值得注意的是，除丁醇外，还可同步生成乙醇与丙酮等副产物[60]。然而，该途径存在高还原力需求、氧敏感性强以及反应可逆性较强等问题，导致异源宿主中的正丁醇产量较低。支链氨基酸分解代谢途径（branched-chain amino acid catabolism， BCAA catabolism）作为微生物代谢网络的重要模块之一，广泛分布于梭菌、酿酒酵母等微生物中。该途径不仅是合成异丁醇、3-甲基-1-丁醇等支链高级醇的核心合成路径，还兼具代谢稳态调控和碳流定向分配的双重生理功能[61]。在氨基酸代谢途径中支链氨基酸经转氨酶催化生成相应α-酮酸；α-酮异戊酸在丙酮酸脱氢酶复合体作用下脱羧形成异丁酰-CoA；通过醛脱氢酶（Ald6）与醇脱氢酶（Adh2）的协同催化，最终生成目标高级醇。

二元醇的生物合成通过天然代谢途径的工程化改造与非天然人工途径的创新设计，显著提升了目标产物的合成效率与工业化潜力。1，3-丙二醇的工业化生产依赖甘油脱水酶途径，杜邦公司通过动态调控NADH再生系统与副产物代谢分流，实现产量135 g/L、生产效率3.5 g/(L·h)的突破性进展[62]。同时，针对维生素B_{12}依赖性限制，研究

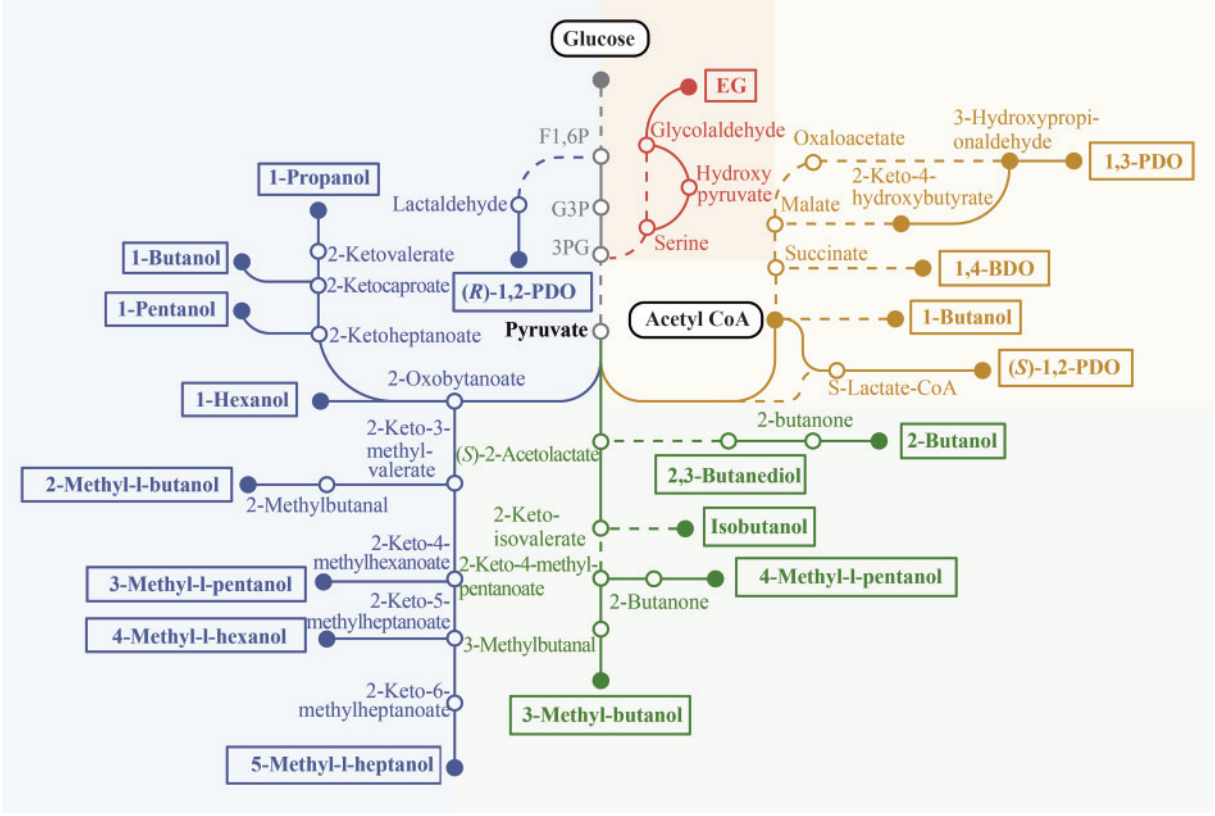


图4 葡萄糖、乙酰辅酶A合成高级醇

[EG—乙二醇；1,3-PDO—1,3-丙二醇；1,4-BDO—1,4-丁二醇；(*R*)-1,2-PDO—*R*-1,2-丙二醇；(*S*)-1,2-PDO—*S*-1,2-丙二醇；Acetyl-CoA—乙酰辅酶A；S-Lactate-CoA—琥珀酰-乳酸辅酶A]

Fig. 4 Synthesis of higher alcohols from glucose and acetyl-CoA

[EG—Ethylene glycol; 1,3-PDO—1,3-Propanediol; 1,4-BDO—1,4-Butanediol; (*R*)-1,2-PDO—(*R*)-1,2-Propanediol; (*S*)-1,2-PDO—(*S*)-1,2-Propanediol; Acetyl CoA—Acetyl coenzyme A; S-Lactate-CoA—Succinyl-lactate coenzyme A]

者开发了高丝氨酸途径与3-羟基丙酸途径等非天然合成路线，其中Li团队[63]通过整合多底物协同利用模块，将产量提升至11.21 g/L，验证了非天然代谢网络的工程可行性。2,3-丁二醇通过混合酸发酵途径合成，酿酒酵母敲除乙醇脱氢酶基因（*ADH*）后产量达178 g/L[64]，谷氨酸棒杆菌优化转氢酶基因（*udhA*）后（2*R*,3*R*）-BDO产量达144.9 g/L[65]。1,4-丁二醇的合成以Genomatica公司设计的琥珀酰辅酶A路径为代表，通过琥珀酸半醛脱氢酶（SucD）和辅酶A转移酶（Cat2）协同作用，结合副产物基因敲除（*ΔadhE*，*Δldh*），产量达125 g/L，年产超10万吨[66]；1,5-戊二醇的合成依赖赖氨酸衍生途径，Cen等[67]通过戊二胺中间体转化，补料发酵产量达9.25 g/L。

表1列出了微生物生产各种高级醇的研究进展。

表1 微生物生产高级醇的进展

Table 1 Advances in the microbial production of higher alcohols

产物 Products	宿主 Hosts	基因类型（敲除；过表达） Genotypes (knockout; overexpression)	底物 Substrates	发酵条件 Fermentation conditions	产量/(g/L) Titer/(g/L)	参考文献 References
n-PrOH	*Escherichia coli*	*ΔilvA ΔilvB*; *kivD adh2 cimA*^{mut} *leuABCD*	Glucose	Shake flask	2.78	[68]
	E. coli	*ΔlacI ΔlysA ΔmetA ΔtdhA ΔiclR ΔilvIH ΔilvBNΔrpoS thrA*^{C1034T} *lysC*^{C1005T}	Glycerol	Bioreactor (Fed-batch)	10.3	[69]
IPA	*E. coli*	None; *lacI*^{q} *thl atoDA adc adhB-593*	Glucose	Stirred flask	143	[70]
	E. coli	None; *thl atoDA adc adhB-593 bgl-blc*	Cellobiose	Shake flask	4.1	[71]
	E. coli	None; *thl ctfAB adc adhB-593*	Glucose	Shake baffled flask	13.6	[72]

续表

产物 Products	宿主 Hosts	基因类型（敲除；过表达） Genotypes (knockout; overexpression)	底物 Substrates	发酵条件 Fermentation conditions	产量/(g/L) Titer/(g/L)	参考文献 References
Alcohol mixture	*Clostridium acetobutylicum*	*Δbuk:: ermC*; *adc ctfAB adhB-593*	Glucose	Bioreactor	35	[73]
	C. acetobutylicum	*Δbuk ΔC1502*; *adc ctfAB adhB-593*	Glucose	Bioreactor	20.4	[74]
n-BuOH	*Synechococcus elongatus*	None; *atoB, hbd, crt, ter, AdhE2*	CO_2	Flask and bioreactor	0.015	[75]
	S. elongatus	None; *nphT7 phaB aphaJ ter bld yqhD*	CO_2	Static capped flask	0.029 9	[76]
	Clostridium tyrobutyricum	*Δpta Δbuk*; $adhE^{D485G}$	Glucose	Bioreactor	130	[77]
	C. tyrobutyricum	None; *adhE2*	Mannitol	Bioreactor; anaerobic	20.5	[78]
	S. cerevisiae	None; *thl, hbd, crt, bcd, etfAB, adhE2*	Galactose	Shake flask	0.002 5	[79]
	Pseudomonas putida	None; *thl, hbd, crt, bcd, etfAB, adhE2*	Glycerol	Shake flask	0.024	[80]
	P. putida	None; *thl, hbd, crt, bcd, etfAB, adhE1*	Glycerol	Shake flask	0.122	[80]
	Lactobacillus brevis	None; *thl, hbd, crt, bcd, etfAB*	Glucose	Vial	0.3	[81]
	Clostridium ljungdahlii	None; *thlA, hbd, crt, bcd, adhE, and bdhA*	Syngas	Shake flask	0.148	[82]
	E. coli	*ΔldhA ΔadhE ΔfrdBC Δpta*; *atoB hbd crt adhE2 fdh ter*	Glucose	Bioreactor; anaerobic	30	[83]
IBOH	*E. coli*	*ΔadhE ΔldhA ΔfrdBC Δfnr Δpta ΔpflB*; *alsS ilvCD kivD adh2*	Glucose	Static capped flask	22	[8]
	E. coli	None; *alsS ilvCD kivD adhA*	Glucose	Bioreactor;	56	[84]
	S. elongatus	None; *alsS, ilvCD, kivd, yqhD*	CO_2	Bottle	0.450	[85]
	Corynebacterium crenatum	None; *ILV2, ILV3, ILV5, kivD, ADH6*	Lignocellulose	Bottle	5.61	[86]
	Bacillus megaterium	None; *kivd, yqhD*	Glucose	Test tube	0.3	[87]
	Bacillus subtilis	*Δldh*; *ilvCD alsS kivD adh2*	Glucose	Bioreactor	3.83	[88]
	Clostridium cellulolyticum	None; *kivD yqhD alsS ilvCD*	Cellulose	Not specified	0.66	[89]
	Corynebacterium glutamicum	*ΔaceE Δpqo ΔilvE ΔldhA Δmdh*; *ilvBNCD pntAB kivD adhA*	Glucose	Bioreactor	13	[90]
	Ralstonia eutropha	*ΔphaB2C2 ΔphaC1AB1*; *alsS ilvCD kivD yqhD*	CO_2	Bioreactor with electrodes	0.09	[91]
	R. eutropha	*ΔphaCAB ΔilvE ΔbkdAB ΔaceE*; *adh ilvBHCD kivD*	Fructose	Shake flask	0.27	[92]
	S. cerevisiae	*Δlpd*; *kivD ADH6 ILV2 ILV5c ILV3c ILV2C MAE1*	Glucose	Shake flask	1.62	[93]
	Zymomonas mobilis	None; *kdcA Pgap als ilvC ilvD*	Glucose	Shake flask	4	[94]
	Clostridium thermocellum	*Δhpt*; *kivd, alsS, ilvBN, ilvCD, ilvD*	Cellulose	Consolidated bioprocessing	5.4	[95]
	C. glutamicum	*ΔilvE*; *ilvBNC, ilvD, adhA, kivd*	Glucose	Shake flask	20.8	[96]
2-BuOH	*Lactobacillus diolivorans*	None; *pduQ, PDO-DH (NAD(P)H)*	*meso*-2,3-butanediol/glycerol	Bioreactor	13.4	[97]
	Klebsiella pneumoniae	*ΔldhA*; $pduCDEGH^{Q337A\ or\ F375I}$, *adh*	Glucose	Shake flask	1.03	[98]

续表

产物 Products	宿主 Hosts	基因类型（敲除；过表达） Genotypes (knockout; overexpression)	底物 Substrates	发酵条件 Fermentation conditions	产量/(g/L) Titer/(g/L)	参考文献 References
2-BuOH	*L. brevis*	None; None	*meso*-2,3-butanediol	Test tube	0.88	[99]
	Lactobacillus buchneri	None; None	butaNone	Test tube	0.04	[99]
	S. cerevisiae	None; *pduCDEGH, adh*	*meso*-2,3-butanediol	Shake flask	0.004	[100]
	Lactobacillus spp.	None; None	*meso*-2,3-butanediol	Shake flask	0.41	[101]
2M1B	*E. coli*	*ΔmetA Δtdh*; *ilvGM ilvCD ilvA kivD adh2 thrABC*	Glucose	Shake baffled flask	1.25	[7]
	Brevibacterium flavum	None; *ilvC-ilvD-alsS, kivd-ADH2*	Glucose; duckweed	Shake flash	19.5/17.5	[102]
	C. crenatum	None; *Cgl1271, Cgl1273, Cgl1268, kivd*; *adh2*	Glucose	Shake flash	5.26	[103]
3M1B	*E. coli*	None; *alsS ilvCD kivD adh2 leuA^{G462D} leuBCD*	Glucose	Shake flask	9.5	[104]
	B. flavum	None; *ilvC-ilvD-alsS, kivd-ADH2*	Glucose; duckweed	Shake flask	0.79/0.78	[102]
	C. crenatum	None; *Cgl1271, Cgl1273, Cgl1268, kivd*; *adh2*	Glucose	Shake flask	3.78	[103]
2,3-BDO	*Serratia marcescens*	*swrI, swrR, slaA, slaB, slaC, slaR*; *swrR*	Glucose	Shake flask	42.5	[105]
	S. marcescens	*ΔswrW*; *swrW*	Sucrose	Fed-batch	152	[106]
	Z. mobilis	*ΔPDC*; *LlkivD, ARO10, THI3, ADH6, ADH7, PpIlv2, PpIlv6, PpIlv5, PpIlv3, ScATF1*	Glucose	Shake flask	13.3	[107]
	E. coli	None; *budB, budA, budC*	Glucose	Shake flask	*meso*-BDO,17.7	[108]
	E. coli	None; *alsS, alsD, bdhA from K. pneumoniae*	Glucose	Shake flask	(*R*,*R*)-BDO,5.8	[109]
	E. coli	None; *alsS, alsD, adh from C. beijerinckii*	Glucose	Shake flask	(*R*,*R*)-BDO,5.1	[109]
	E. coli	None; *alsS, alsD, adh from T. brockii*	Glucose	Shake flask	(*R*,*R*)-BDO,6.1	[109]
	E. coli	None; *budA, budB, ydjL*	Glucose	Shake flask	(*S*,*S*)-BDO,2.2	[110]
	E. coli	None; *budA, budB, ydjL*	Glucose	Shake flask	0.66	[111]
	E. coli	None; *budA, budB, ydjL*	Glucose	Bioreactor	(*R*,*R*)-BDO,30.5	[112]
	E. coli	*ΔldhA, ΔpflB, ΔadhE, ΔlpdA::K.p.lpd E354 K, Δmdh, ΔarcA*; *gltAR164L, ilvBN, aldB, bdh1*	Glucose	Bioreactor	88	[113]
	E. coli	None; *budA, budB, budC*	Glucose	Bioreactor High oxygen	52.1	[114]
	E. coli	*ΔldhA, ΔadhE, Δpta, ΔfrdA*; *budA, budB, budC*	Glucose	Bioreactor Low oxygen	68.1	[114]
	E. coli	*ΔldhA, Δpta, ΔadhE, ΔpoxB*; *alaS, alsD, budC*	Glucose	Shake flask	*meso*-BDO,14.5	[115]
	E. coli	None; *bdh, fdh*	Diacetyl	Bioreactor	(*S*,*S*)-BDO,31.7	[116]
	E. coli	None; *budC, bdh*	Diacetyl	Shake flask	(*S*,*S*)-BDO,2.2	[117]
	E. coli	None; *budA, budB, budC*	Sugar beet molasses	Fed-batch	56.2	[114]
	E. coli	*ΔfrdABCD, ΔldhA, ΔadhE, ΔlpdA, Δpta*; *budB, budA, budC*	Algal hydrolysate	Shake flask	*meso*-(*S*,*S*)-BDO,14.1	[118]

续表

产物 Products	宿主 Hosts	基因类型（敲除；过表达） Genotypes (knockout; overexpression)	底物 Substrates	发酵条件 Fermentation conditions	产量/(g/L) Titer/(g/L)	参考文献 References
2,3-BDO	*E. coli*	*ΔpoxB, ΔldhA, ΔackA, Δpta*; *alsS, alsD, budC, ced3A*	Cellodextrin	Shake flask	*meso*-BDO,5.5	[119]
1,3-PDO	*E. coli*	*ΔgldA, ΔglpK, ΔaldA, ΔaldB, ΔmgsA, ΔptsHI,* replacing *gapA* promoter with the synthetic short *1.5 GI* promoter (SEQ ID NO:28); *galP*	Glucose	Bioreactor	112	[120]
	E. coli	None; *gpd1-gpp2* fusion gene, *dha operon, rpoS*	Glucose	Bioreactor	12.1	[121]
	E. coli	None; *dhaB, yqhD, gdrA, gdrB, fdh1, gapN, galP, glk*	Glycerol	Shake flask	13.47	[122]
	E. coli	None; *dhaB1, dhaB2, yqhD*	Glycerol	Bioreactor	104.4	[123]
	E. coli	None; *dhaB1, dhaB2, dhaT*	Glycerol and glucose	Bioreactor	41.7	[124]
	Corynebacterium glutamicum	*Δald, Δpyk, Δadh, ΔpoxB, ΔldhA, Δppc, Δzwf*; *hdpA-gldA, gpd1, gpp2, yqhD, pduCEDGH*Downregulation: *gapA*	Glucose and xylose	Fed-batch	110.4	[125]
	Vibrio natriegens	*Δpta-ackA, ΔarcA, ΔadhE, ΔaldB, Δldh, Δpfl, ΔsthA, ΔglpR, ΔaldA, ΔfrdABCD*; *pntAB, phaP*	Glycerol	Fed-batch	69.5	[126]
	E. coli	*ΔthrB*;*yqhD, lysC, ser*$^{CR42W/R77W}$, *metL, pdc*	Glucose	Fed-batch	3.03	[127]
	E. coli	None; *mcrC, pduP, mcrN, yqhD, prpE*	Glucose; Xylose, Glycerol, Acetate	Shake flask; Fed-batch	2.93; 7.98	[63]
	E. coli	*ΔlysC, ΔgltA*; *ppc*	Glucose	Shake flask; Fed-batch	6.41; 11.21	[128]
	E. coli	*ΔglpK, ΔptsG*; *yqhD, pntAB, galP, glk*	Isoprene	Shake flask	2.5	[129]
1,3-BDO	*E. coli*	None; *phaAB, bld*	Glucose	Fed-batch	15.76	[130]
	E. coli	*Δldh, Δpta, ΔackA, ΔadhE*; *bld*^{L273T}, *yqhD, phaAB, pntAB*	Glucose	Optimized fermentation	13.40	[131]
	E. coli	*Δldh, Δpta, ΔackA, ΔadhE*; *phaAB, yqhD, pntAB, car, sfp*	Glucose	Optimized fermentation	0.40	[131]
	E. coli	*Δzwf, Δedd, ΔpfkA, ΔpfkB*; *pk, glpX, thl, hbd, tesB, car*	Glucose	Fed-batch	22.66	[132]
	E. coli	*ΔadhE, ΔpoxB, ΔldhA, Δpta-ackA, ΔatoB, ΔtesB, ΔyciA*; *phaAB, bld, yqhD*	Glucose	Fed-batch	23.13	[133]
	E. coli	*ΔadhE, ΔpoxB, ΔldhA, ΔyciA, ΔpdhR, Δpgi, ΔgntR*; *phaAB, bld, yjgB, zwf*	Glucose	Fed-batch	71.1	[134]
	E. coli	*Δpta, ΔyjgB, ΔadhE, ΔldhA, ΔpflB, ΔadhP, ΔyqhD, ΔeutG, ΔilvB, ΔpoxB*; *AKR, DERA, PDC*	Acetaldehyd; 3-Hydroxy-butanal (3-HB)	Biotransformation	2.4	[135]
1,4-BDO	*E. coli*	*Δsad::cat2-bld-bdh, ΔlacZ::cat1-sucD 4hbd, ΔllpdA::K.p.lpdA D354KΔpflB, ΔarcA, Δmdh, ΔadhE, ΔldhA,* knock down *tesB*; *gabD, ybgC, gltAR163L*	Glucose	Bioreactor	1.8	[136]
	E. coli	*ΔadhE, ΔldhA, ΔpflB, Δmdh, ΔarcA, lpdA::K.p.lpdD354K*; *gltAR163L, sucA, 4hbd, cat2, ald, adh*	Glucose	Bioreactor	18	[66]

续表

产物 Products	宿主 Hosts	基因类型（敲除；过表达） Genotypes (knockout; overexpression)	底物 Substrates	发酵条件 Fermentation conditions	产量/(g/L) Titer/(g/L)	参考文献 References
1,4-BDO	*E. coli*	*ΔaraA, Δicd*; *araC, araD, araA, araB, araE, kivd, yqhd*	L-Arabinose	Bioreactor	1.51	[137]
	E. coli	None; None	Glucose	Commercial-scale fermentation	>125	[138]
	E. coli	None; *gadB, gabT, yqhD, car, ppc, gltA*^{R163L}	Amino acids (AAs), Glucose	General metabolic platform	1.41	[139]
	E. coli	*ΔyagE, ΔxylA, ΔyjhH*; *Kvid*^{V461I}, *xylBCDX, yqhD*	Glucose, Xylose	D-xylose, L-arabinose, D-galacturonate	12	[137]
	E. coli	*ΔuxaC, ΔgarL, Δicd*; *udh, garD, ycbC, xylA(CC), kivd, yqhd*	D-Galacturo-nate	Bioreactor	16.5	[137]
	E. coli	*ΔxylA, ΔyjhH, ΔyagE, Δicd*; *xylB, xylC, xylD, xylX, xylA(CC), kivdV461I, yqhd*	Xylose	Bioreactor	12	[137]
1,2-PDO	*E. coli*	*ΔpoxB, ΔfrdA, ΔmgsA, ΔadhE::pdcD*; *gldA, mgs*	Glucose	Shake flask	0.7	[140]
	E. coli	*ΔlldD::mmsB, ΔackA-pta::pct, ΔldhA::Lldh*; *pct, pdcD, mmsB*	Glucose	Shake flask	1.04	[4]
	E. coli	*ΔldhA::KanR*;*mgs, gldA, fucO*	Glucose	Bioreactor	4.5	[141]
	E. coli	*Δzwf, ΔtpiA, ΔgloA, ΔldhA, ΔadhE,*; *mgsA, gldA, fucO*	Glucose	Shake flask	5.13	[120]
	E. coli	*ΔlldD, Δdld, ΔldhA, ΔadhE*; *pct, pduP, yahK*	D-/L-Lactate	Shake flask	(*R*)-1,2-PDO,1.5; (*S*)-1,2-PDO,1.7	[142]
	E. coli	*Δac kA-pta, ΔldhA, ΔdhaK*; *dhaKL, gldA, mgsA, yqhD*	Glycerol	Bioreactor	5.6	[143]
BT	*E. coli*	*ΔyiaE, ΔycdW, ΔxylA KivD (Lactococcus lactis)*; None	Xylose	Fed-batch, carbon flux optimization	10.03	[144]
	E. coli	None; *YqhD, YjhG*	Xylose	Optimized cultivation	5.1	[145]
	E. coli	*ΔxylA, ΔxylB, ΔyjhE, ΔyagH, ΔycdW*; *xdh, mdlC*	Xylose	Fed-batch	2.38	[146]
	E. coli	None; *XylD, KdcA (Lactococcus lactis), AdhP (E. coli)*	Xylose	Optimized cultivation	5.1	[147]
	E. coli	*ΔfadE*; *XylD, fadD*	Xylose, free fatty acids	Fed-batch	1.1 (BT esters)	[148]
	S. cerevisiae	None; *XylB, XylD*	Xylose, rice straw hydrolysate	Aerobic fermentation	1.7	[149]

在代谢网络优化方面，研究者通过多维度技术策略提升合成效率。首先，基于CRISPR-Cas系统重构嗜盐菌的类脂肪酸延长途径与戊糖磷酸途径，强化了碳流导向高级醇合成的效率[39]。其次，整合代谢物响应型启动子优化苯乙烯衍生途径与Ehrlich途径，并协同调控磷酸烯醇式丙酮酸（phosphoenolpyruvate，PEP）代谢流，使2-苯乙醇产量提升至2.36 g/L[150]。此外，利用代谢通量平衡分析协同改造脱氧酮糖酸途径（Entner-Doudoroff，ED）与EMP途径，实现了异丁醇合成路径的能效最大化，产量达280 mmol/L，产率84%[96]。

4 代谢工程优化策略

在代谢工程领域，优化策略的核心在于通过系统性改造细胞代谢网络，提升目标产物的合成

效率与产量。目前研究聚焦于三大关键方向，辅因子优化、竞争途径去除与酶优化策略，旨在解决代谢通量分配、资源竞争及催化效率等核心瓶颈问题。例如辅因子循环与供给不足，辅因子再生效率低导致NAD(P)H稳态失衡，竞争途径的隐性分流加剧碳源浪费，酶的底物特异性不足限制催化通量等。这些问题可以通过系统性整合基因编辑、蛋白质工程及动态调控技术解决，显著优化代谢网络的全局效率。

4.1 辅因子优化

在高级醇生物合成过程中伴随着大量的氧化还原反应，辅因子在氧化还原反应中扮演着至关重要的角色。例如正丁醇的天然CoA依赖型途径通常需要4分子NADH，而异丁醇、2-甲基-1,3-丁二醇（2-methyl-1,3-butanediol， 2MB）、3-甲基-1,2-丁二醇（3-methyl-1,2-butanediol，3MB）等支链高级醇的合成途径需要2分子NAD(P)H[151]。

目前，辅因子工程主要优化策略为以下四种：①重建辅因子合成；②提升辅因子的代谢水平；③平衡辅因子的稳态；④增加辅因子的活性形式[152]。其中，代谢工程策略通过引入外源基因或改造内源途径，重建辅因子的生物合成途径，满足高级醇合成对辅因子的需求。Wang等[153]在*E. coli*中通过将谷氨酸脱氢酶与转氨酶和醇脱氢酶耦合，使得共底物α-酮戊二酸（α-ketoglutaric acid）和NAD(P)H可同时再生，实现辅因子的自给自足，从而将2-苯乙醇（2-PE）的生物催化效率提高了3.8倍。Liu等[154]在*S. cerevisiae*中通过引入具有NAD^+依赖性以及反馈抗性的TyrA突变体，使羟基酪醇的产量达到1.12 g/L，较基础菌株提高了36.9%。同样，通过过表达关键酶或优化代谢途径，提升细胞内辅因子的代谢水平，可以增强还原力供给。Lian团队[155]通过组合代谢工程策略以及增加关键辅助因子3′-磷腺苷-5′-磷酸（3′-phosphoadenosine-5′-phosphosulfate， PAPS）的供应，使得硫酸胆固醇（cholesterol sulfate， CS）的产量比毕赤酵母（*Komagataella phaffii*）母株提高了6.8倍以上。在平衡辅因子的稳态方面，通过动态调控辅因子的生成与消耗，维持细胞内辅因子的稳态，避免辅因子竞争或积累导致的代谢抑制[156]。Wang等[157]建立了克雷伯氏菌YT7与厌氧单胞菌（*S.oneidensis*）MR-1的共培养系统，成功替代了外源性电子介体，使1,3-丙二醇的滴度达到了32.30 g/L，增加了185.84%，同时乳酸含量减少了38.82%。同样，可以通过提高辅因子与酶的亲和力或增强辅因子的活性形式，从而提高催化效率。李晗团队[156]通过建立了一个非典型辅因子NMN^+（烟酰胺单核苷酸）的系统，避免了NADH/NAD^+比例失衡导致的副产物积累，通过调控氧化还原平衡在体内和体外均实现了2,3-丁二醇光学纯的高效合成。

4.2 竞争途径去除

除了重新设计合成途径外，删除竞争途径也是提高生物合成效率的核心策略，通过阻断或削弱与目标产物竞争的代谢分支，从而提高目标代谢通量和产物产量。为了降低竞争性消耗、限制目标产物合成，Schwarz等[158]敲除了巴氏梭菌DSM 525中编码氢化酶的*hydA*基因，使在葡萄糖的批次发酵中丁醇浓度比野生型高出5倍。为了减少代谢分流，Sun等[159]在*Klebsiella pneumoniae*中，通过敲除编码丙糖磷酸异构酶的*tpiA*基因，阻断甘油通过糖酵解途径代谢，并过表达*mgsA*和*yqhD*基因，使1,2-丙二醇产量达到9.3 g/L。Kim等[160]在*S. cerevisiae*中通过引入异源2,3-丁二醇生物合成途径，同时删除产生乙醇和甘油的竞争途径，成功地将代谢通量重新导向2,3-丁二醇。Zhu等[4]在*E. coli*$^{BW25113\Delta poxB}$中，通过构建从L-乳酸（L-lactic acid）到*S*-1,2-丙二醇（*S*-1,2-PDO）的人工合成途径，并敲除葡萄糖代谢中的竞争性支路基因，并且破坏主要的碳竞争途径和加强乳酸转化途径，直接从葡萄糖中生产出纯度大于99%的13.7 mmol/L *S*-1,2-丙二醇。副产物的生成会消耗葡萄糖和辅因子，可能抑制目标途径。因此，为减少副产物对生产的影响。Li等[125]在谷氨酸棒状杆菌（*Corynebacterium glutamicum*）中，通过减少有毒的3-羟基丙酸和其他副产物的积累、优化NAD(P)H生成途径和补偿代谢通路等策略来对细胞代谢进行重编程，最终该菌株可同时利用葡

萄糖和木糖，生产98.2 g/L的1,3-丙二醇，单以葡萄糖为底物可生产110.4 g/L的1,3-丙二醇。

4.3 酶优化策略

酶的改造与设计是优化代谢途径的关键手段。酶作为代谢途径中的核心催化剂，决定了反应速率和底物转化效率。通过定向进化和理性设计来提高酶的催化效率、调控酶的表达量，可以增加目标化合物的产量，进一步确保了代谢通量的高效运行。Huo团队[161]利用氨基酸响应型转录衰减子*ivbL*和高级醇响应型转录激活因子BmoR构建了自动动态调节系统（CRUISE），该系统通过过表达*ivbL*和BmoR分别调控相关酶表达，形成反馈激活模式使氨基酸的流量在合成与转化途径间动态平衡，成功将异丁醇产量提高至40.4 g/L（图5）。提升酶在极端条件（高温、pH波动、有机溶剂等）下的稳定性，可以延长酶的催化寿命。Liao团队[162]对乳酸乳球菌（*Lactococcus lactis*）的2-酮异戊酸脱羧酶（Kivd）进行了热稳定性优化。研究通过构建一个随机突变文库（约8000个独立变体），最终获得最优变体LLM4。同野生型相比，LLM4变体的熔点提高了13 ℃，在60 ℃预孵育1 h后的残留活性提高10.5倍，半衰期提高4倍。这一改造提高了异丁醇等酮酸衍生醇类的工业化应用潜力。Volker Sieber等[163]通过对乳酸乳球菌的酮酸脱羧酶（KdcA）进行了优化，成功提高了热稳定性和异丁醇耐受性。使得突变体7M.D在50 ℃、4%异丁醇环境下的稳定性提高了600余倍，并在9%（体积分数）异丁醇中仍然保持大于20%的活性。这一改造使7M.D更适用于嗜热菌（>65 ℃）和无细胞系统中的高级醇生产，提高了在高温条件下的工业应用潜力。通过设计不依赖昂贵辅因子的酶变体，可以减少或替换酶对辅因子（如NADH、维生素B_{12}）的依赖。Zhang团队[124]通过过表达酪酸梭菌（*Clostridium butyricum*）的维生素B_{12}非依赖型甘油脱水酶dhaB1及其激活因子dhaB2并构建共培养体系，最终在10 L生物反应器中，1,3-丙二醇产量达到41.65 g/L。通过启动子工程、核糖体结合位点（RBS）优化或基因拷贝数调整，可以增强酶的表达量。Huang团队[112]在*E. coli*中利用不含*lac*序列的pUC6S载体，构建了人工合成基因簇，并利用启动子P01驱动*R*,*R*-2,3-丁二醇脱氢酶编码基因（*ydjL*），最终在18 h内以80 g/L葡萄糖生产了30.5 g/L *R*,*R*-2,3-丁二醇，且对映体纯度超过99%。

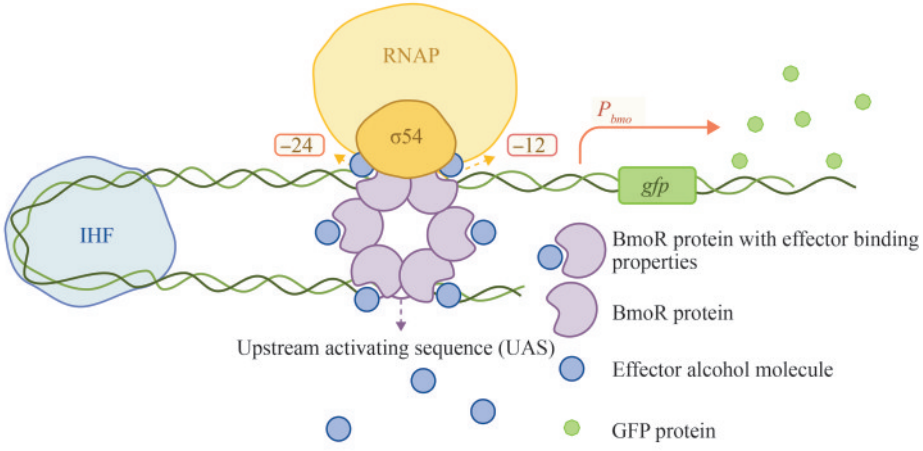


图5 BmoR传感器作用机制

[σ^{54}依赖型启动子P_{bmo}受醇响应蛋白BmoR的调控。当BmoR蛋白感应到醇分子的存在时，会形成多聚体，并结合至启动子P_{bmo}上游的激活序列（UAS）。该激活序列位于σ^{54}-RNA聚合酶结合位点（图中所示为−24和−12区域）上游。由于σ^{54}-RNA聚合酶与BmoR之间存在一定距离，宿主整合因子（IHF）蛋白通过促使DNA形成环状结构，实现远距离的转录调控]

Fig. 5 Mechanism of action for the BmoR sensor

[The σ^{54}-dependent promoter P_{bmo} is regulated by the BmoR protein, which responds to alcohol. When alcohol is present, BmoR forms a multimer (depicted as a hexamer in the figure, a presumed form) and attaches to the upstream activating sequence (UAS) of P_{bmo}, located upstream of the σ^{54}-RNA polymerase binding sites (shown in the figure as the −24 and −12 regions). Due to the distance between the σ^{54}-RNA polymerase and BmoR, DNA looping facilitated by the integration host factor (IHF) protein achieves long-distance regulation.]

5 高级醇生产底盘筛选及优化

5.1 生产菌株耐受性优化策略

在高级醇的生产过程中，初始菌株常因合成途径效率不足及底盘细胞适配性欠佳，难以满足工业化生产要求。由于理性设计耐受性菌株存在较高技术门槛，实验室适应性进化法（adaptive laboratory evolution，ALE）成为优化菌株抗逆能力的首选策略。有研究表明，通过实验室适应性进化在高异丁醇浓度下筛选耐受突变株，结合适应性进化和转录组分析，鉴定出16个与异丁醇耐受相关的基因，优化了菌株的异丁醇耐受性和生产能力。进一步研究发现，当*fadB*或*dppC*过表达时，异丁醇耐受性显著提高，使产量提高了1.5倍[164]。此外，为优化正丁醇生产，研究者倾向于优选天然高耐受菌种替代低效生产菌，目前已筛选出枯草芽孢杆菌（*Bacillus subtilis*）与短乳杆菌（*Lactobacillus brevis*）等耐受3%正丁醇的菌株，酿酒酵母YPS128耐受浓度达4%，而恶臭假单胞菌（*Pseudomonas putida*）甚至可耐受6%的正丁醇[165-168]。

代谢工程手段是提升菌株性能的另一核心路径。通过将合成途径整合至基因组并敲除竞争性代谢分支基因（如乳酸合成基因），可阻断副产物生成并减少碳源损耗[4]。该策略在1,3-丙二醇生产中已获验证，通过构建由L-天冬氨酸经3-羟基丙酸合成1,3-丙二醇的新模块，摇瓶发酵产量达6.41 g/L，补料分批发酵产量提升至11.21 g/L，较优化前提高7.69%[128]。

5.2 高产菌株筛选策略

在绿色生物制造领域，生物传感器已成为高通量筛选与动态调控代谢途径关键工具。通过设计特异性识别元件响应信号分子浓度变化，实时监测代谢状态并快速评估突变体性能，显著提升高产菌株选育效率。目前，该技术已成功应用于小分子化合物及复杂天然产物高产菌株的筛选。利用来自假单胞菌的转录因子BmoR和启动子P_{bmo}构建醇响应系统，BmoR与醇分子结合后可激活P_{bmo}启动子转录[169]。研究显示，通过对BmoR-P_{bmo} σ^{54}依赖性转录因子-启动子对的启动子区域进行定向突变，可扩展生物传感器的动态响应范围，从而优化生物丁醇的合成效率[170]。此外，该系统通过引入稀有密码子标记基因，能够响应胞内氨基酸浓度变化，实现高产氨基酸菌株的精准筛选。同时有研究开发了底物诱导型启动子系统，可实时监测恶臭假单胞菌KT2440的碳源利用效率[171]。

基于上述启动子工程与代谢感应模块的研究基础，可进一步通过整合动态调控单元［如CRISPR干扰（CRISPRi）与反义RNA技术］，通过靶向抑制竞争途径或增强产物外排，实现代谢通量的时空精准控制，从而为高级醇的智能化生物制造提供技术支撑。CRISPR干扰（CRISPRi）与反义RNA技术通过靶向调控代谢网络显著增强了生物传感器系统的功能。CRISPRi利用失活的Cas9蛋白（dCas9）与抑制结构域融合，精准结合基因启动子或调控区域，抑制转录活性以实现动态代谢通量分配[172-181]。例如，通过设计截短的向导RNA，单个向导可同时靶向数百个转录因子结合位点，显著扩展调控范围并揭示非编码元件的功能网络[182]。机器学习模型（如混合效应随机森林）可优化向导RNA设计，预测靶基因沉默效率，进一步提升调控精度[183]。反义RNA通过与靶标mRNA互补配对形成双链RNA（dsRNA），进而激活核输出机制，加速RNA转运至细胞质以促进特定蛋白质的快速合成，从而增强细胞对微环境变化的适应性响应[172]。此外，长非编码RNA（lncRNA）的核输出可能通过类似dsRNA依赖的途径实现，通过调控mRNA稳定性与翻译活性参与细胞应激响应及基因表达动态平衡[172]。通过上述技术创新，生物传感器系统在高产菌株筛选与代谢途径优化中的功能得以显著增强，为生物制造过程的智能化升级提供了重要支撑。

5.3 微生物共培养策略及发酵优化

天然微生物群体在自然界中广泛存在，通过分工协作与代谢互补降低个体代谢负担，并协同应对环境压力。然而，受限于实验室纯培养技术的瓶颈，目前仅有不足1%的微生物可实现单一菌株分离培养[184]。针对此问题，微生物共培养体系通过多菌株代谢网络的协同作用，显著提升了底物利用率与目标产物产量。在大肠杆菌

（*Escherichia coli*）的共培养体系中，通过实验室适应性进化增强了甲羟戊酸的消耗能力，并优化了异戊醇生产，在1∶3（上游∶下游）接种比例下，使用进化菌株的异戊醇产量是亲本菌株的3.3倍[185]。自2014年工程微生物群体（engineering microbial communities，EMC）概念提出以来[186]，研究者开发了多组高效共培养系统：在斯达氏油脂酵母（*Lipomyces starkeyi*）和蜡样芽孢杆菌（*Bacillus cereus*）的共培养体系中，运用POME生物修复法，成功提高了微生物脂质的积累和废水处理效率，共培养体系的最大生物量为（8.89±0.33）g/L，脂质产量为（2.27 ± 0.10）g/L，均显著高于单培养体系[187]。在聚多曲霉（*Aspergillus sydowii*）和枯草芽孢杆菌的共培养体系中，通过整合计算分析工具（MS-DIAL、MS-FINDER）和在线工具（GNPS、MetaboAnalyst），成功鉴定和分析了共培养代谢产物[188]。在大肠杆菌共培养体系中，通过构建不依赖维生素B_{12}的甘油脱水酶（GDHt）表达菌株和过表达1,3-丙二醇氧化还原酶（dhaT）的菌株，提高了1,3-丙二醇的生产效率。结果表明，与单独发酵甘油相比，共发酵甘油和葡萄糖的策略使1,3-丙二醇产量提高了1.3倍。在10 L生物反应器中，最终1,3-丙二醇的产量达到41.65 g/L，生产速率为0.69 g/(L·h)，产率为0.67 mol/mol[124]。此外，蓝藻（*Cyanobacteriota*）与大肠杆菌的共培养系统通过分工协作，由蓝藻固定CO_2供应碳源，大肠杆菌将其转化为二醇类化合物，为低碳生物制造提供了新策略。

在高级醇的生产过程中，产物毒性导致的细胞生长抑制是核心瓶颈。通过优化发酵方式工艺可有效缓解此问题，其中气体剥离法（gas stripping）作为连续发酵的关键技术，已成功应用于工业化生产[189]。采用该技术后，异丁醇的发酵产量从早期的22 g/L的记录突破至50 g/L以上[190]。通过优化共培养体系和发酵方式，可以显著提高高级醇的产量。通过整合共培养体系与工艺优化策略，可显著提升高级醇的产量与经济性。

5.4 基于新技术的优化策略

NGIB通过利用极端微生物（如嗜盐菌、嗜酸菌）作为工程菌株，在开放的非灭菌条件下实现高效生产，显著降低了杂菌污染风险并提升了经济性，同时结合基因组编辑与途径重构技术进一步释放其合成潜力[9-11]。例如，最新研究开发的耐盐微生物平台——分离自超级蠕虫（*Zophobas atratus*）肠道的济州杆菌（*Jejubacter* sp. L23），经多维度工程改造后，可利用非粮生物质菊粉为底物，在非灭菌条件下高效合成异丁醇、*R*,*R*-2,3-丁二醇及番茄红素等大宗化学品，实现在3 L规模的非无菌发酵过程中，异丁醇产量可达41 g/L，充分体现了NGIB的高效性与环保优势[191]。在极端嗜热古菌（*Pyrococcus furiosus*）中引入AOR-Adh途径，结合合成气发酵技术，不仅验证了高温环境下长链醇合成的可行性，而且通过跨物种途径移植与极端条件适配，展现了NGIB在复杂环境中的技术延展性[192]。微生物代谢途径的动态调控是平衡生长与生产的关键策略，通过重构代谢物响应型启动子、生物传感器或群体感应系统，实现基因表达与代谢通量的自主调节。例如，基于代谢物浓度的启动子设计可实时感知胞内信号，而群体感应系统则通过细胞密度依赖的调控机制优化产物合成[193-194]。

计算机辅助设计技术的快速发展为代谢工程提供了新范式。通过整合代谢组、蛋白质组与转录组等多组学数据，计算工具可解析底盘细胞代谢网络并设计全新合成路径。例如，利用BNICE算法构建1,4-丁二醇的生物合成途径，并结合OptKnock对宿主菌株的代谢目标进行优化，从而显著提升产物得率，使1,4-丁二醇产量最高可达18 g/L[66]。此外，利用ORACLE平台对大肠杆菌1,4-丁二醇合成途径进行风险分析与优化，提出关键酶表达调控策略[195]；利用CRISPR-COPIES工具非靶向搜索快速定位基因组最佳整合位点，加速基因回路构建[196]。值得关注的是，AlphaFold 3模型的发布为蛋白质结构与功能预测提供了更高精度的解决方案，通过深度学习预测酶的三维构象与活性位点，为理性设计高效催化元件（如脱羧酶、醇脱氢酶）提供了分子基础，从而优化高级醇合成途径的碳流分配与能量效率[197]。

6 总结与展望

利用废弃生物质生产高级醇作为可再生能源，

是实现循环经济与可持续发展的重要路径。其环境价值体现在对农业废弃物、林业副产物及城市有机垃圾的资源化利用，可显著减少传统焚烧或填埋导致的温室气体排放与土壤污染，同时替代化石燃料降低碳排放强度。经济层面，生物质原料的低成本与广泛分布为规模化生产提供了基础，而高级醇的高附加值则增强了产业盈利能力。技术创新如微生物代谢工程与酶催化优化、政策激励包括碳税减免与生物基补贴，以及市场机制涵盖绿色认证和碳交易，共同构成三螺旋发展模型。合成生物学改造的梭菌属菌株使丁醇合成效率提升30%，欧盟强制掺混政策则推动生物燃料市场年增长8.2%，而产业链协同效应例如玉米芯联产乙醇与木糖醇，使综合利润率突破25%[14-16]。

然而，产业化进程仍受制于原料预处理成本占生产总成本的40%，政策标准波动如欧盟认证壁垒，以及技术转化周期长等挑战。技术瓶颈集中于原料预处理与转化效率，纤维素类生物质的复杂结构需通过高温酸解或超临界流体处理实现降解，但高能耗与设备腐蚀问题推升成本。经济障碍则体现在全产业链成本上，原料收集、运输的分散性导致物流体系复杂。政策与法规约束亦不容忽视，例如部分国家对生物燃料的掺混比例设限，且废弃物处理标准趋严，增加合规成本。此外，生态与社会风险需审慎评估。大规模生物质种植可能引发与粮食作物的土地竞争，而生产过程中若废水处理不当，易造成水体富营养化。为突破上述瓶颈，技术创新与系统优化是关键方向。NGIB通过合成生物学改造微生物代谢途径，可提升产物选择性及产率。同时，耦合热化学转化（如催化气化）与生物发酵的联产工艺，可提高原料利用率并降低能耗。政策与市场协同亦不可或缺，政府需制定长期补贴机制与碳税优惠政策；而市场需建立绿色认证体系，引导消费者选择低碳产品。未来，可以通过多维度策略整合包括技术创新降本、政策激励扩容、公众意识提升，废弃生物质高级醇有望成为能源转型的核心支柱。这不仅将缓解化石能源依赖，更可推动农业废弃物资源化与乡村经济振兴，最终实现环境效益与产业发展的双重目标。

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