Acetate: A New Feedstock for Biomanufacturing

Abstract

The imperative to transition from fossil-derived feedstocks to renewable carbon sources has catalyzed intensive research into alternative substrates for industrial biomanufacturing. Acetate, a simple two-carbon (C2) organic acid, has emerged as a promising next-generation feedstock that challenges the traditional dominance of sugar-based bioprocessing. This review critically examines the potential of acetate as a building block for biomanufacturing value-added chemicals, biofuels, and biomaterials. We analyze the diverse sources of acetate production—from syngas fermentation and lignocellulosic biomass degradation to electrochemical CO_2 reduction—and evaluate the metabolic pathways that enable microbial conversion of this substrate. Key challenges including acetate toxicity, low carbon efficiency, and thermodynamic constraints are discussed alongside recent metabolic engineering strategies to overcome these limitations. We compare acetate-based bioprocessing with conventional sugar fermentation, highlighting both advantages and persistent bottlenecks. Finally, we provide a forward-looking perspective on how integration of electrochemistry, synthetic biology, and process intensification may position acetate as a cornerstone feedstock for the circular bioeconomy within the next decade.

1. Introduction: The Case for Alternative Carbon Sources

The global bioeconomy has historically relied on first-generation feedstocks—primarily glucose and sucrose derived from food crops—to produce chemicals, fuels, and materials through microbial fermentation[1]. However, this paradigm faces mounting challenges: competition with food security, agricultural land constraints, and the limited sustainability of monoculture farming systems[2]. The escalating climate crisis has further intensified the search for carbon-negative or carbon-neutral production routes that can utilize waste streams, atmospheric CO_2, or non-food biomass.

C1 (CO, CO_2, methane, formate, methanol) and C2 (acetate, ethanol) substrates have garnered significant attention as next-generation feedstocks due to their natural abundance, potential for low-cost production from waste materials, and independence from arable land[3]. Among these alternatives, acetate occupies a unique position. As one of the simplest carboxylic acids and a central metabolite in cellular biochemistry, acetate serves as a direct precursor to acetyl-CoA—the universal biosynthetic hub for numerous valuable compounds including fatty acids, polyketides, isoprenoids, and amino acids[4][5].

The economic viability of acetate as a feedstock is compelling. Current market prices for acetate (USD 350-450/ton) are comparable to or lower than glucose (USD 500/ton), particularly when acetate is derived from waste streams where production costs may be negative[6][7]. Moreover, acetate can be generated through multiple sustainable routes: anaerobic digestion of organic waste, dark fermentation of lignocellulosic biomass, syngas fermentation from gasified materials, and increasingly, through electrochemical reduction

of CO_2 using renewable electricity[8][9][10]. This diversity of production pathways decouples acetate supply from seasonal agricultural cycles and geographic constraints.

Despite these advantages, acetate-based biomanufacturing faces formidable challenges. Acetate exhibits concentration-dependent toxicity to most industrial microorganisms, disrupting pH homeostasis and membrane integrity at concentrations as low as 5-10 g/L[11][12]. The carbon efficiency of acetate bioconversion is inherently lower than glucose due to ATP consumption during activation and the need for additional reducing power[13]. Furthermore, the relatively dilute nature of acetate in typical production streams (often <30 g/L) poses downstream processing challenges[14].

This review synthesizes recent advances in acetate biomanufacturing across multiple dimensions: (i) diversification of acetate production technologies, (ii) elucidation of acetate metabolism and tolerance mechanisms, (iii) metabolic engineering strategies for enhanced acetate utilization and product synthesis, (iv) bioprocess optimization and scale-up considerations, and (v) techno-economic and environmental assessments. We critically evaluate competing approaches, identify persistent knowledge gaps, and articulate a vision for how acetate may transform industrial biotechnology in the coming decade.

2. Sources of Acetate: From Waste to Wealth

2.1 Biochemical Routes: Fermentative Acetate Production

Acetate is a ubiquitous end-product of anaerobic metabolism across diverse microbial communities. Dark fermentation of carbohydrate-rich biomass by mixed microbial consortia readily produces acetate alongside other volatile fatty acids (VFAs), with typical concentrations ranging from 5-20 g/L[15]. Lignocellulosic biomass—the most abundant renewable organic material on Earth—represents a particularly attractive feedstock for acetate generation. During thermochemical or enzymatic pretreatment of lignocellulose, acetyl groups attached to hemicellulose are released as acetic acid, typically comprising 2-5% of dry biomass weight[16].

Syngas fermentation has emerged as a promising technology for acetate production from gasified materials. Acetogenic bacteria such as *Moorella thermoacetica* and *Clostridium* species can convert syngas (mixtures of CO, CO_2, H_2, and N_2) into acetate via the Wood-Ljungdahl pathway with high selectivity[17][18]. Recent pilot-scale demonstrations have achieved acetate titers exceeding 25 g/L from biomass-derived syngas, though challenges remain regarding gas impurities (H_2S, HCN, tars) that inhibit microbial activity[19]. Mobile pilot plants coupling gasification with fermentation have validated this integrated approach at industrially relevant scales, providing critical data for techno-economic modeling.

Anaerobic digestion (AD) of organic waste represents another established route for acetate generation, particularly during the acidogenesis phase before methanogenesis. By interrupting the AD process or employing methanogen inhibitors, acetate can be selectively accumulated to 10-30 g/L[20]. This approach offers dual benefits: waste valorization and production of a useful chemical feedstock. Municipal wastewater, food processing residues, and agricultural waste have all been explored as feedstocks for this purpose.

2.2 Electrochemical Routes: CO_2-to-Acetate Conversion

The electrochemical reduction of CO_2 to acetate using renewable electricity represents a paradigm shift with profound implications for carbon capture and utilization[21][22]. Two main strategies have been developed: direct electrochemical CO_2 reduction and tandem electrolysis combining CO_2-to-CO conversion followed by CO-to-acetate reduction[23].

Direct CO_2 electroreduction to acetate requires formation of C-C bonds, a process that remains challenging due to competing reactions producing C1 products (CO, formate) and other C2 compounds (ethylene, ethanol)[24]. Recent advances in copper-based catalysts functionalized with amine-terminated dendrimers have improved acetate selectivity by modulating the local microenvironment to stabilize key intermediates such as adsorbed CO and ethenone[25][26]. However, selectivities typically remain below 50% and current densities are modest compared to C1 products.

The tandem electrolysis approach has shown more promise for scale-up. A recent kilowatt-scale demonstration coupled a 500 cm^2 CO_2 electrolyzer (0.40 kW) with a 1,000 cm^2 CO electrolyzer (0.71 kW), producing 98 L of 1.2 M acetate at 96% purity over 125 hours of continuous operation[27][28]. Techno-economic analysis suggests production costs of 79 ¢/kg for acetic acid, potentially decreasing to 35 ¢/kg with optimized auxiliary processes and available tax credits—competitive with the current market price of 68 ¢/kg[29]. This represents a critical milestone demonstrating the technical and economic feasibility of electrochemical acetate production at scales approaching industrial relevance.

Microbial electrosynthesis (MES) represents a hybrid approach combining electrochemistry with biology. In MES systems, electroautotrophic microorganisms accept electrons from a cathode to reduce CO_2 to acetate via the Wood-Ljungdahl pathway[30][31]. H_2-mediated electron transfer using low-cost nickel foam cathodes has achieved acetate titers up to 16 g/L in mixed-culture systems[32]. MES offers advantages of biological specificity and mild reaction conditions, though challenges of electrode biofouling, low volumetric productivity, and scale-up complexity remain to be addressed[33].

2.3 Integration with Existing Bioprocesses

A frequently overlooked source of acetate is overflow metabolism during high-density microbial fermentations. *Escherichia coli* and many industrial microorganisms excrete acetate when carbon flux exceeds the capacity of central metabolism, particularly under aerobic high-glucose conditions[34]. This acetate accumulation is typically viewed as problematic, causing growth inhibition and reduced product yields. However, innovative bioprocess designs are beginning to view this as an opportunity: acetate produced in one fermentation stage can be captured and fed to engineered strains optimized for acetate consumption in a subsequent stage, creating integrated multi-stage biorefinery concepts[35].

3. Metabolic Pathways and Cellular Responses to Acetate

3.1 Acetate Assimilation Pathways in Model Organisms

Understanding how microorganisms metabolize acetate is fundamental to rational engineering of acetate-utilizing cell factories. In *E. coli*, the primary model organism for industrial biotechnology, two major pathways activate acetate to acetyl-CoA[36][37]:

1. **The AckA-Pta pathway:** Acetate kinase (AckA) and phosphotransacetylase (Pta) catalyze a reversible two-step conversion. Acetate is first phosphorylated to acetyl-phosphate (consuming ATP), then converted to acetyl-CoA. This pathway predominates at higher acetate concentrations (>10 mM or ~0.6 g/L) and benefits from lower ATP expenditure compared to alternative routes[38][39].
2. **The Acs pathway:** Acetyl-CoA synthetase (Acs) directly converts acetate to acetyl-CoA in an ATP-dependent reaction (consuming ATP and producing AMP and pyrophosphate). This pathway is more active at low acetate concentrations (<10 mM) and is subject to complex post-translational regulation[40].

Wild-type *E. coli* grows efficiently on acetate concentrations between 2.5-50 mM (0.15-3 g/L)[41]. Deletion studies reveal functional specialization: Δ*acs* mutants grow poorly below 10 mM acetate, while Δ*pta-ackA* mutants show impaired growth above 25 mM, indicating that the two pathways serve complementary concentration ranges[42].

Upon entering central metabolism as acetyl-CoA, acetate carbon flows primarily through the glyoxylate shunt—a modified tricarboxylic acid (TCA) cycle that bypasses the decarboxylation steps[43]. The glyoxylate shunt, comprising isocitrate lyase (AceA) and malate synthase (GlcB), enables net synthesis of C4 intermediates from C2 units, providing biosynthetic precursors while avoiding CO_2 loss. Upregulation of this pathway is essential for efficient growth on acetate as the sole carbon source[44].

Recent work has identified alternative acetate activation pathways with potential biotechnological applications. In *Geobacter sulfurreducens*, a succinyl-CoA:acetate CoA-transferase (SCACT) encoded by *atoAD* genes catalyzes acetate activation without ATP consumption by coupling it to the TCA cycle intermediate succinyl-CoA[45][46]. Heterologous expression of this pathway in *Shewanella oneidensis* enhanced acetate consumption rates by ~35% and improved bioelectricity production in microbial fuel cells[47]. This ATP-independent route presents significant advantages for energy-limited bioprocesses and is being explored in multiple host organisms.

3.2 The Acetate Toxicity Problem

A major barrier to industrial acetate utilization is concentration-dependent growth inhibition[48][49]. The undissociated form of acetic acid (pKa = 4.76) readily crosses cell membranes and dissociates in the near-neutral cytoplasm, releasing protons that acidify the intracellular environment[50]. This disrupts pH homeostasis, collapses the proton-motive force, and diverts ATP consumption toward maintaining intracellular pH via proton efflux systems[51].

At the molecular level, acetate stress triggers pleiotropic cellular responses. Transcriptomic analyses reveal upregulation of stress response genes including heat shock proteins (DnaK, GroEL), oxidative stress defenses (catalases, peroxidases), and acid resistance systems[52][53]. The cell envelope is particularly vulnerable: acetate increases membrane permeability by altering lipid composition, specifically decreasing unsaturated fatty acids and increasing cyclopropane fatty acids[54].

Acetate concentrations above 5-10 g/L dramatically reduce growth rates and cell viability in most industrial microorganisms, severely limiting process productivity[55]. Even acetate-producing organisms such as acetogens exhibit product inhibition, with fermentations stalling at 10-20 g/L acetate depending on the strain and cultivation conditions[56]. This toxicity threshold is problematic because economically viable bioprocesses typically require product concentrations exceeding 50-100 g/L to minimize downstream separation costs.

3.3 Natural and Engineered Tolerance Mechanisms

Understanding acetate tolerance mechanisms has become a priority research area. Adaptive laboratory evolution (ALE) studies have identified multiple genetic determinants of acetate tolerance[57]. In *Clostridium* sp. AWRP, adaptation to 10 g/L acetate resulted in three point mutations in genes encoding electron-bifurcating hydrogenases and *dnaK*, accompanied by broad transcriptional reprogramming that shifted metabolism from autotrophy toward utilization of organic substrates[58].

A particularly intriguing tolerance mechanism involves polyhydroxybutyrate (PHB) mobilization. Introduction of a stress-induced PHB mobilization pathway in *E. coli* increased cell viability under 0.6 g/L acetic acid from 25% to 53%[59]. Mechanistic studies revealed that PHB mobilization modulates membrane fatty acid composition, increasing the ratio of cyclopropane to unsaturated fatty acids by 35%, thereby reducing membrane fluidity and permeability to acetic acid[60]. This strategy enabled co-production of succinate and PHB from acetate, demonstrating practical applications.

Rewiring global regulatory networks represents another strategy. Error-prone PCR mutagenesis of the global regulator cAMP receptor protein (CRP), which controls ~444 genes in *E. coli*, identified a D138Y mutation that increased growth rate in 15 g/L acetate by more than 5-fold[61]. Transcriptional profiling revealed differential expression of over 400 CRP-regulated genes, including those involved in the TCA cycle and phosphotransferase system, highlighting the polygenic nature of acetate tolerance[62].

Rational engineering approaches have focused on overexpressing acetate utilization pathways to accelerate conversion before toxic concentrations accumulate. Overexpression of *acs* or *ackA-pta* combined with glyoxylate shunt enzymes (*aceA*, *glcB*) and citrate synthase (*gltA*) has yielded strains with improved acetate consumption rates[63][64]. However, the energetic burden of high pathway flux must be balanced against the benefits of rapid detoxification.

4. Metabolic Engineering for Acetate-Based Bioproduction

4.1 Acetyl-CoA-Derived Chemicals: Natural Targets

The strategic advantage of acetate as a feedstock is its direct conversion to acetyl-CoA, the central biosynthetic precursor for a vast array of valuable compounds. Several product categories have been successfully demonstrated from acetate, each with distinct challenges and achievements.

Polyhydroxyalkanoates (PHAs): These biodegradable bioplastics are naturally produced from acetyl-CoA via sequential condensation reactions. PHB production from acetate has been extensively studied in *E. coli*[65]. Comparative analysis of glucose, ethanol, and acetate as carbon sources revealed that engineered *E. coli* expressing a mutant alcohol

dehydrogenase (AdhE A267T/E568K) produced 3.12 g/L PHB from ethanol (48.7% of CDW), significantly higher than from glucose or acetate alone[66]. The superior performance with ethanol reflects more favorable NADH generation without additional ATP consumption. Nevertheless, acetate remains attractive for PHB production when derived from waste streams, with yields of 0.40 g PHB/g acetate reported[67].

Isoprenoids: The mevalonate pathway, which synthesizes isoprenoid precursors from acetyl-CoA, has been engineered for production from acetate. Phloroglucinol (PG), a platform chemical for pharmaceuticals and polymers, was produced from acetate in *E. coli* through overexpression of ACS and pathway optimization[68]. Similarly, mevalonate titers of several grams per liter have been achieved from acetate in engineered strains, though productivities remain lower than glucose-based processes[69].

Higher alcohols: Isobutanol, a promising biofuel with superior properties compared to ethanol, has been produced from acetate by engineering pyruvate biosynthesis. Recent work expressing pyruvate-ferredoxin oxidoreductase (YdbK) alongside the isobutanol synthesis pathway achieved 157 mg/L isobutanol from 50 mM acetate—the highest titer reported from acetate as sole carbon source in batch cultivation[70][71]. This milestone demonstrates feasibility but highlights the substantial gap remaining before commercial viability.

Organic acids: Itaconic acid, succinate, and other platform chemicals derived from TCA cycle intermediates represent logical targets for acetate bioconversion. A screen identified an acetate-tolerant *E. coli* strain that, after overexpressing *cis*-aconitate decarboxylase and enhancing glyoxylate shunt flux, produced 3.57 g/L itaconic acid from acetate with 16.1% yield[72]. Succinate production from acetate reached 2-3 g/L in strains combining acetate tolerance mechanisms (PHB mobilization) with optimized biosynthetic pathways[73].

Diols: 2,3-Butanediol and acetoin production from acetate was achieved through systematic metabolic engineering in *E. coli*[74]. Overexpression of the entire acetate utilization pathway increased acetate consumption rate by 15% (to 0.78 g/g/h) and diol titer by 35% (to 1.16 g/L). Comparison of alternative acetate uptake routes revealed that the AckA-Pta pathway outperformed Acs for diol production, likely due to lower ATP expenditure and thermodynamic regulation[75].

4.2 Expanding the Product Portfolio Beyond Acetyl-CoA Derivatives

While acetyl-CoA-derived products represent the most direct targets, metabolic engineering has expanded acetate utilization to compounds requiring additional biosynthetic steps.

Fatty acid-derived products: Lipids and fatty acid ethyl esters (biodiesel precursors) have been produced from acetate using oleaginous yeasts. *Yarrowia lipolytica* cultivated on dilute acetate in semicontinuous fermentation accumulated triacylglycerols with optimized carbon-to-nitrogen ratios[76]. This approach addresses the challenge of dilute acetate streams from anaerobic digestion, though lipid titers (typically <10 g/L) remain below those achieved with glucose.

Amino acids: Production of amino acids from acetate requires anaplerotic reactions to provide biosynthetic precursors. Engineering of phosphoenolpyruvate (PEP) and pyruvate biosynthesis pathways has enabled amino acid production, though at low titers[77].

Specialty chemicals: Triacetic acid lactone (TAL), a versatile platform chemical for plastics and food ingredients, was produced from xylose and acetate co-utilization in

engineered *Saccharomyces cerevisiae*[78]. This work demonstrated complete utilization of both lignocellulose-derived substrates, converting acetate from a toxic byproduct into a valuable co-substrate that boosts efficiency.

4.3 Comparative Carbon and Energy Efficiency

A critical question for acetate-based biomanufacturing is how carbon and energy efficiency compare to conventional glucose fermentation. Thermodynamic analysis reveals fundamental constraints[79]:

Feedstock	Carbon Recovery	ATP Generation	Reducing Power
Glucose	High (90-95%)	+++ (glycolysis)	+++ (glycolysis + TCA)
Ethanol	100% (to acetyl-CoA)	++ (via AdhE)	++ (NADH generation)
Acetate	100% (to acetyl-CoA)	- (ATP consumed)	- (no net generation)

Table 1: Comparison of feedstock utilization efficiency

Acetate activation consumes ATP (1 ATP via AckA-Pta or 2 ATP equivalents via Acs), and no reducing power (NADH, NADPH) is generated during this process[80]. In contrast, glucose catabolism via glycolysis generates both ATP and NADH before reaching acetyl-CoA. This fundamental energetic disadvantage means that additional acetate must be oxidized through the TCA cycle solely to provide ATP and reducing equivalents needed for biosynthesis[81].

The carbon efficiency penalty depends on the target product. For products requiring only carbon (e.g., PHAs), theoretical yields from acetate can approach those from glucose. However, for products requiring substantial reducing power (e.g., higher alcohols, alkanes), a significant portion of acetate carbon is inevitably lost as CO_2 during oxidative metabolism to generate NADH/NADPH[82]. This explains why observed acetate-to-product yields are typically 30-50% lower than glucose-based processes for the same product.

Metabolic flux analysis comparing acetate and glucose utilization in *E. coli* producing PHB revealed that acetate requires 40% more carbon input to achieve equivalent biomass and product formation[83]. This carbon penalty is partially offset when acetate is obtained from waste streams at negative cost, but it constrains maximum achievable yields and necessitates larger fermentation volumes.

5. Bioprocess Design and Scale-Up Considerations

5.1 Fed-Batch and Continuous Cultivation Strategies

Bioprocess design for acetate-based fermentations must navigate the narrow window between sufficient substrate concentration for productivity and inhibitory levels that impair cell growth[84]. Fed-batch strategies that maintain acetate concentration below toxicity thresholds (typically 5-10 g/L) are commonly employed, though this compromises volumetric productivity[85].

Continuous cultivation with cell retention offers advantages for acetate-based processes. By maintaining steady-state acetate concentrations and preventing accumulation, continuous systems can operate at higher dilution rates than batch processes[86]. Microbial electrosynthesis studies demonstrated that continuous mode with hydraulic retention time (HRT) of 3 days achieved acetate production rates of 652 ppm/day with improved pH control compared to fed-batch operation[87].

Two-stage cultivation separating growth and production phases has shown promise. Cells are first cultivated on a preferred substrate (e.g., glucose) to build biomass, then transferred to acetate-containing medium for product formation[88]. This approach avoids the slow growth rates characteristic of acetate utilization but requires careful process integration.

5.2 Co-Substrate Utilization: Synergistic Approaches

Co-feeding acetate with complementary carbon sources can overcome energetic limitations while fully utilizing available substrates. Engineered *S. cerevisiae* capable of co-utilizing xylose and acetate from lignocellulosic hydrolysates achieved complete substrate consumption with higher product titers than either substrate alone[89]. The xylose provided energy and reducing power while acetate contributed additional carbon flux through acetyl-CoA.

Similarly, glucose-acetate co-feeding has been explored where glucose provides energy for ATP generation and biosynthetic precursors (via glycolysis), while acetate supplies additional acetyl-CoA units directly[90]. Optimal co-feeding ratios depend on the target product and must be determined empirically, balancing carbon catabolite repression effects that may suppress acetate utilization in the presence of preferred sugars[91].

5.3 In Situ Product Recovery and Process Intensification

Given the relatively low product titers typical of acetate-based fermentations, in situ product recovery (ISPR) strategies are critical for economic viability[92]. Extractive fermentation using organic solvents or adsorbent resins can remove inhibitory products and acetate itself during fermentation, enabling higher conversions[93].

For volatile products such as ethanol or butanol, gas stripping ISPR has been integrated with acetate fermentations, though careful optimization is needed to avoid excessive evaporation of acetate at acidic pH[94]. Membrane-based separation, including pervaporation and electrodialysis, shows promise for selective product removal while retaining cells and substrates[95].

Biofilm reactors and immobilized cell systems can enhance volumetric productivity by increasing cell density while providing tolerance to acetate stress[96]. Recent work engineering biofilm formation in *Shewanella* through enhanced c-di-GMP signaling demonstrated improved acetate consumption and bioelectricity generation in microbial fuel cells[97].

5.4 Pilot-Scale Demonstrations and Industrial Implementation

The transition from laboratory to industrial scale reveals challenges invisible at small scale. The most advanced demonstrations exist for acetate production rather than downstream bioconversion. The pilot-scale tandem CO_2 electrolyzer producing acetate at kilowatt scale represents a critical proof-of-concept for electrochemical routes[98]. For gas fermentation, mobile pilot plants coupled to biomass gasifiers have produced >25 g/L acetate from syngas, validating integrated biorefinery concepts[99].

However, pilot demonstrations of acetate-to-chemical bioconversions remain scarce. The longest continuous fermentations reported are measured in weeks rather than months, and most studies employ defined media rather than complex acetate-containing industrial streams[100]. Impurities in real feedstocks—residual lignin derivatives from lignocellulose, trace metals from waste streams, variable pH and composition—all impact process robustness[101].

Scale-up calculations for acetate-based processes reveal challenging economics. Assuming 50 g/L product titer (optimistic for current acetate-utilizing strains) and 0.3 g product/g acetate yield, producing 10,000 tons/year of product requires fermenting ~67,000 tons/year of acetate. At typical volumetric productivities of 0.5 g/L/h, this demands ~2,500 m^3 of fermentation capacity operating continuously[102]. These volumes are manageable for large-scale facilities but exceed the capacity of distributed, small-scale biorefineries that might be co-located with waste acetate sources.

6. Techno-Economic Assessment and Sustainability Analysis

6.1 Cost Structure and Economic Drivers

Techno-economic analyses of acetate-based biorefineries reveal that feedstock cost and downstream separation dominate process economics when acetate is purchased at market price[103]. However, when acetate is derived from waste valorization (negative feedstock cost), the economics shift favorably. Key cost components include:

- **Feedstock:** Variable from -$50/ton (waste-derived) to +$450/ton (chemical-grade)
- **Fermentation:** Capital costs for large reactors, utilities (pH control, aeration), and nutrients
- **Downstream processing:** Separation costs scale inversely with product titer; distillation energy for volatile products
- **Wastewater treatment:** Acetate residues require biological or chemical oxygen demand removal

For electrochemical acetate production, power cost is the dominant expense, comprising >50% of total production cost[104]. Reducing cell voltage and improving Faradaic efficiency could decrease acetate production costs from 79 ¢/kg to 35 ¢/kg, below the current market price of 68 ¢/kg, but this requires continued advances in electrocatalyst performance and system integration[105].

The value proposition for acetate-based biomanufacturing is strongest when:

1. Acetate is obtained from waste streams at low or negative cost

2. Target products command high value (pharmaceuticals, specialty chemicals rather than bulk fuels)
3. Processes are integrated with existing infrastructure (e.g., wastewater treatment plants, biorefineries) to minimize capital investment

6.2 Life Cycle Assessment: Carbon Footprint and Environmental Impact

Life cycle assessment (LCA) studies comparing acetate-derived versus fossil-derived chemicals reveal significant environmental benefits when acetate originates from waste or CO_2[106]. Key findings include:

- Acetate from CO_2 electrolysis using renewable electricity exhibits carbon intensity of 0.2-0.8 kg CO_2-eq/kg acetate, compared to 2-3 kg CO_2-eq/kg for petrochemical acetic acid via methanol carbonylation[107]
- Syngas fermentation-derived acetate from biomass gasification shows 50-70% lower greenhouse gas emissions than fossil comparators, depending on biomass source and energy inputs[108]
- Waste-derived acetate through anaerobic digestion avoids methane emissions while producing useful feedstock, offering carbon-negative pathways when coupled to renewable energy[109]

However, LCA results are highly sensitive to system boundaries and allocation methods. When additional processing (concentration, purification) is required to convert dilute acetate streams into fermentation-grade feedstock, environmental benefits diminish substantially[110]. Energy-intensive downstream separations to recover dilute products can negate upstream carbon savings.

6.3 Policy Landscape and Market Drivers

Regulatory frameworks and policy incentives significantly influence the economic viability of acetate-based biomanufacturing. Carbon pricing mechanisms, renewable fuel standards, and tax credits for carbon capture and utilization (CCU) technologies can shift the economic calculus[111]. In the United States, the 45Q tax credit ($50-85/ton CO_2) and the Inflation Reduction Act provisions for clean hydrogen and chemicals provide substantial subsidies that improve project economics[112].

The European Union's Renewable Energy Directive and circular economy action plan create market pull for bio-based products from waste feedstocks[113]. This regulatory environment favors acetate-based processes that utilize organic waste, particularly when demonstrated at commercial scale.

Corporate sustainability commitments by major chemical producers create demand for drop-in bio-based chemicals with lower carbon footprints[114]. Several companies have announced investments in gas fermentation and CO_2 electrolysis technologies, signaling growing industrial interest despite remaining technical challenges.

7. Emerging Frontiers and Unconventional Applications

7.1 Acetate in Photosynthetic Organisms and Plant Cell Cultures

An unexpected frontier is the use of acetate as a supplementary carbon source for photosynthetic organisms. Traditionally, photosynthesis provides both carbon and energy for plant and algal growth. However, engineering acetyltrophic capability into photoautotrophs could enable "mixotrophic" cultivation with enhanced productivity[115].

Recent work demonstrated that tobacco plant cell cultures can incorporate exogenous acetate into proteins and carbohydrates, though concentrations above 8 mM inhibit growth when combined with sucrose[116]. Lower acetate concentrations (2-4 mM) support modest biomass increases without sucrose, but endogenous glyoxylate cycle enzyme expression is too low for efficient acetate utilization[117]. This suggests that substantial metabolic engineering—introducing or upregulating glyoxylate shunt enzymes—will be necessary to enable robust acetate utilization in plant cells.

The concept of "electro-agriculture" proposes using electrochemically derived acetate to support plant growth independent of photosynthesis[118]. Theoretical models suggest that plants engineered to utilize acetate as the primary carbon source could reduce agricultural land requirements by 88% if cultivated in vertical indoor farms powered by renewable electricity[119]. While highly speculative, this vision illustrates the potentially transformative applications of acetate if biological and engineering challenges are overcome.

Similarly, algal cultivation supplemented with acetate could enhance lipid or protein production beyond what photosynthesis alone provides. Genetic modifications enabling efficient acetate uptake and assimilation in microalgae are under development, with potential applications in food production, aquaculture feed, and biofuel synthesis[120].

7.2 Acetate-Based Bioelectrochemical Systems

Beyond microbial electrosynthesis for acetate production, acetate serves as an electron donor in bioelectrochemical systems including microbial fuel cells (MFCs) and microbial electrolysis cells (MECs)[121]. Acetate oxidation at bioanodes provides electrons for electricity generation or chemical reduction at the cathode, enabling sustainable wastewater treatment coupled with energy or chemical production[122].

Recent advances in exoelectrogenic bacteria—microorganisms capable of transferring electrons to solid electrodes—have improved power densities and coulombic efficiencies[123]. Engineering acetate catabolic pathways to enhance electron flux toward extracellular electron transfer (EET) systems has increased bioelectricity output by >2-fold[124]. Optimized MFC systems fed with acetate-rich wastewater achieve power densities approaching 10 W/m^2, though economic viability remains marginal[125].

An intriguing application is the use of acetate as an electron shuttle in syntrophic microbial communities. In these systems, one organism ferments complex substrates to acetate, which is then consumed by acetogens or methanogens, facilitating complete waste mineralization while capturing energy[126]. Engineering such consortia for stability and productivity represents a frontier in community biotechnology.

7.3 Non-Model Organisms and Extremophiles

While *E. coli* dominates academic research, industrial implementation may benefit from alternative hosts with native advantages for acetate utilization[127]. Several candidates are under investigation:

- **Acetogenic clostridia:** Natural acetate producers via the Wood-Ljungdahl pathway, these organisms can be reverse-engineered to consume acetate for product synthesis. Their native tolerance and efficient acetate metabolism offer advantages, though genetic tractability lags behind *E. coli*[128].
- **Pseudomonas species:** Known for versatile carbon source utilization and acetate tolerance, *Pseudomonas putida* has been engineered for production of medium-chain-length PHAs and other chemicals from acetate[129].
- **Corynebacterium glutamicum:** This industrial workhorse for amino acid production has been engineered for acetate utilization, leveraging its robust central metabolism and existing industrial infrastructure[130].
- **Extremophiles:** Thermophilic and halophilic acetate-utilizing microorganisms offer advantages for integrated processes. Thermophiles enable operation at elevated temperatures compatible with thermal pretreatment of lignocellulose, while halophiles tolerate high ionic strength, reducing contamination risk and enabling product recovery through salting-out effects[131][132].

7.4 Cell-Free Systems and Enzyme Cascades

Cell-free synthetic biology offers an alternative to whole-cell fermentation by reconstructing metabolic pathways in vitro using purified enzymes[133]. For acetate bioconversion, cell-free systems eliminate toxicity constraints and enable precise control over pathway flux[134]. Recent demonstrations include:

- Cell-free synthesis of mevalonate from acetyl-CoA using reconstituted mevalonate pathway enzymes with continuous cofactor regeneration[135]
- In vitro fatty acid synthesis from acetyl-CoA and malonyl-CoA using fatty acid synthase multienzyme complexes[136]

While productivity and economics currently limit cell-free systems to high-value products, continued cost reductions in enzyme production and cofactor recycling technologies may expand applicability[137]. Hybrid approaches combining whole-cell acetate activation with cell-free product synthesis represent an unexplored opportunity.

8. Persistent Challenges and Knowledge Gaps

Despite significant progress, fundamental and applied challenges constrain the widespread implementation of acetate-based biomanufacturing.

8.1 Incomplete Understanding of Acetate Tolerance Mechanisms

While various tolerance strategies have been identified, a comprehensive systems-level understanding of acetate stress and adaptation remains elusive[138]. Key questions include:

- What are the relative contributions of membrane composition, proton efflux systems, metabolic rerouting, and transcriptional regulation to overall tolerance?

- Can tolerance mechanisms identified in different organisms be combined synergistically?
- How does acetate toxicity interact with other fermentation stresses (osmotic pressure, temperature, product inhibition)?

Multi-omic approaches integrating genomics, transcriptomics, proteomics, metabolomics, and fluxomics across diverse acetate-tolerant strains could reveal conserved mechanisms and species-specific adaptations[139]. Machine learning applied to these datasets may identify non-intuitive engineering targets.

8.2 Low Volumetric Productivity

Even with optimized strains, acetate-based fermentations typically exhibit volumetric productivities 5-10-fold lower than equivalent glucose-based processes[140]. This productivity gap translates directly to larger capital investment for fermentation capacity. Contributing factors include:

- Slow growth rates on acetate (~0.1 h^{-1} vs. ~0.5 h^{-1} on glucose)
- Low maximum cell densities before toxicity effects dominate
- Limited carbon flux through biosynthetic pathways due to energetic constraints
- Suboptimal enzyme kinetics when pathway components are not evolved for high acetate flux

Addressing this challenge may require not only strain engineering but also novel bioreactor designs (high cell density systems, biofilm reactors, perfusion culture) and process intensification strategies[141].

8.3 Product Diversity Limitations

The range of products successfully produced from acetate, while expanding, remains limited compared to the hundreds of chemicals synthesized from glucose in industrial biotechnology[142]. Products requiring biosynthetic precursors distant from acetyl-CoA (e.g., aromatic amino acids from the shikimate pathway) face particularly low yields due to extensive metabolic rerouting requirements.

Expanding the acetate-derived product portfolio requires:

- Engineering of anaplerotic and cataplerotic reactions to provide diverse biosynthetic precursors
- Synthetic pathways that minimize CO_2 loss and maximize carbon retention
- Cofactor balancing strategies to address NADH/NADPH deficits inherent to acetate metabolism

8.4 Feedstock Quality and Consistency

Real-world acetate-containing feedstocks exhibit high variability in concentration, pH, ionic strength, and contaminant profiles[143]. Syngas-derived acetate may contain residual CO, H_2S, and tars; lignocellulose-derived acetate includes lignin derivatives and furfurals; anaerobic digestion produces acetate alongside other VFAs and ammonia. Robust industrial strains must tolerate this complexity, yet most metabolic engineering is performed in defined laboratory media.

Closing this gap requires:

- Characterization of industrially relevant feedstock compositions
- High-throughput screening and evolution using real feedstocks
- Engineering of tolerance mechanisms for specific contaminant classes
- Upstream purification processes that balance cost and purity

8.5 Integration Across Scales and Disciplines

Acetate-based biomanufacturing is inherently interdisciplinary, requiring integration of electrochemistry (for CO_2 reduction), chemical engineering (for gasification and syngas cleanup), microbiology (for fermentation), and process engineering (for downstream separations)[144]. Few research groups or companies possess expertise across all these domains, hindering development of fully integrated systems.

Successful commercialization will require collaborative frameworks that bridge disciplinary boundaries and support end-to-end technology demonstration from feedstock to final product at industrially relevant scales[145].

9. Future Perspectives: Acetate in the 2030 Bioeconomy

9.1 Near-Term Outlook (2025-2030)

Over the next five years, acetate-based biomanufacturing will likely advance through incremental improvements rather than revolutionary breakthroughs. Expected developments include:

Niche applications with favorable economics: Production of high-value specialty chemicals (pharmaceutical intermediates, food additives, fine chemicals) from acetate will reach commercial demonstration, leveraging waste-derived acetate to offset low yields and productivities.

Process integration in existing biorefineries: Capture and utilization of acetate byproducts from lignocellulosic ethanol or biochemical production facilities will be implemented, adding revenue streams and improving overall carbon efficiency without requiring stand-alone acetate biorefineries.

Continued scale-up of electrochemical acetate production: Demonstration plants at the 10-100 kW scale for CO_2-to-acetate electrolysis will validate long-term stability and provide rigorous techno-economic data for investment decisions.

Expansion of genetic toolkits: CRISPR-based genome editing, advanced biosensors, and high-throughput screening platforms will be adapted for acetate-utilizing non-model organisms, accelerating strain development timelines.

9.2 Medium-Term Transformation (2030-2035)

By the mid-2030s, if current trajectories continue, acetate could become established as a legitimate feedstock for certain chemical sectors:

Integrated bio-electrochemical refineries: Facilities coupling renewable electricity-driven CO_2-to-acetate conversion with biological upgrading to chemicals will emerge, particularly in regions with abundant renewable energy and carbon capture infrastructure[146]. These facilities will represent a new industrial archetype: the "electrorefinery" combining electrolysis, fermentation, and chemical processing.

Commodity chemical production: At least one acetate-derived commodity chemical (likely a biodegradable polymer, solvent, or platform acid) will achieve market penetration at the 10,000-100,000 ton/year scale, demonstrating economic viability and establishing supply chains.

Synthetic microbial consortia: Engineered multi-species communities performing complex metabolic transformations (e.g., lignocellulose → acetate → product) in single reactors will advance beyond laboratory curiosities to pilot demonstrations, leveraging natural division of metabolic labor.

Policy-driven adoption: Strengthening carbon pricing and renewable chemical mandates will create economic incentives that tip marginal acetate-based processes into profitability, particularly in the European Union and California.

9.3 Long-Term Vision (2035 and Beyond)

Looking toward 2040 and beyond, several transformative scenarios could position acetate as a cornerstone of the bioeconomy:

Decoupling food production from chemical feedstocks: If acetate from CO_2 electrolysis becomes cost-competitive with sugar crops, the current competition between food and chemical/fuel production could be dramatically reduced. Agricultural land currently devoted to industrial crops could be returned to food production or ecological restoration[147].

Distributed biomanufacturing: Small-scale, modular acetate-producing electrolyzers powered by local renewable electricity could enable distributed chemical production, reducing transportation costs and increasing supply chain resilience. This model contrasts with the centralized megascale facilities that dominate current chemical industry.

Carbon-negative chemical production: Acetate derived from atmospheric CO_2 capture coupled with renewable energy enables fundamentally carbon-negative production pathways for chemicals. If biomass from regenerated forests or perennial crops is also converted to acetate via gasification, total system carbon balance could be substantially negative[148].

Synthetic biology convergence: The distinction between electrochemical and biological systems may blur as hybrid bio-electronic organisms are developed—microbes engineered with heterologous electron transfer systems that directly reduce CO_2 to products via extracellular electron uptake, bypassing acetate altogether[149]. Alternatively, artificial cells combining synthetic biology with nanomaterials could perform acetate-based synthesis with efficiencies exceeding natural organisms.

Agricultural transformation through electro-agriculture: Though highly speculative, the vision of food crop plants engineered to utilize acetate as primary carbon source could revolutionize agriculture if technical barriers (efficient acetate uptake, glyoxylate cycle engineering, regulatory approval) are overcome. This would enable food production independent of climate, in urban vertical farms or even extraterrestrial habitats[150].

9.4 Critical Uncertainties

Several factors will determine whether acetate achieves transformative impact or remains a niche feedstock:

Energy transition speed: Acetate's competitiveness via electrochemical routes depends critically on renewable electricity costs. Delayed renewable energy deployment would slow adoption.

Breakthrough strain engineering: Revolutionary advances in tolerance, productivity, or product scope (e.g., through AI-designed enzymes, synthetic chromosomes, or xenobiological systems) could dramatically accelerate timelines. Conversely, if current engineering paradigms hit fundamental limits, progress may stagnate.

Competing C1 feedstocks: Methanol, formate, and CO are also under intensive development as non-food feedstocks. If one of these alternatives achieves breakthrough performance, it could capture investment and infrastructure development, path-dependent advantages that slow acetate adoption.

Regulatory and social acceptance: Novel production pathways, especially those involving genetic engineering or synthetic biology, face uncertain regulatory timelines and variable public acceptance across geographies. Regulatory bottlenecks could delay commercialization by years or decades in some markets.

Black swan technologies: Transformative developments in chemical synthesis (e.g., low-temperature electrochemical routes for commodity chemicals), artificial photosynthesis, or direct air capture with mineralization could disrupt the entire premise of bio-based chemical production.

10. Conclusions

Acetate has emerged from relative obscurity as a mere fermentation byproduct to claim attention as a potentially transformative feedstock for sustainable chemical production. Its advantages are compelling: independence from food crops, diverse sustainable production routes from waste and CO_2, direct metabolic connection to central biosynthetic intermediates, and attractive economics when derived from negative-cost feedstocks. Yet formidable challenges remain: acute cellular toxicity, inherent energetic inefficiency, low productivity, limited product diversity, and immature downstream processing.

The acetate-based biomanufacturing landscape in 2024 is one of vigorous research activity, expanding strain engineering capabilities, and advancing scale-up demonstrations, particularly for electrochemical acetate production. Multiple viable pathways exist—syngas fermentation, lignocellulose pretreatment liquors, CO_2 electrolysis, and microbial electrosynthesis—each with distinct technical maturity, cost structures, and optimal applications.

Critical to future progress is a system-level perspective that integrates microbial physiology, metabolic engineering, process design, and techno-economics. Acetate will not displace glucose as the dominant fermentation feedstock in the near term; rather, it will complement existing technologies in applications where its unique advantages outweigh its limitations. The most promising near-term opportunities lie in waste valorization,

production of high-value specialty chemicals, and integration into existing biorefineries as a supplementary feedstock.

Looking ahead, the role of acetate in the bioeconomy will be shaped by progress on multiple fronts: systems-level elucidation of tolerance mechanisms enabling rational engineering, development of robust industrial strains through accelerated evolution and synthetic biology, demonstration of integrated electrochemical-biological systems at commercial scales, and supportive policy frameworks that value carbon utilization and circular economy principles.

If these challenges are addressed systematically, acetate has the potential to become a major pillar of sustainable chemical manufacturing by 2035-2040—not as the sole next-generation feedstock, but as one element of a diversified portfolio that includes C1 compounds, lignocellulose, and potentially even direct biological CO_2 fixation. The transition from fossil-derived to bio-based chemical production is not a binary switch but a gradual diversification of feedstocks and technologies. Acetate's ultimate contribution will be determined by continued innovation, strategic investment, and sustained commitment to sustainability as a primary industrial imperative.

The challenges are substantial, but so too is the promise: a chemical industry that recycles carbon, utilizes waste, operates on renewable energy, and produces without competing for food. Acetate may well be one of the keys to unlocking this vision.

References

[1] Lee, S. Y., & Kim, H. U. (2015). Systems strategies for developing industrial microbial strains. *Nature Biotechnology*, 33(10), 1061-1072.

[2] Ragauskas, A. J., et al. (2006). The path forward for biofuels and biomaterials. *Science*, 311(5760), 484-489.

[3] Liew, F., et al. (2022). Microbial utilization of next-generation feedstocks for the biomanufacturing of value-added chemicals and food ingredients. *Frontiers in Bioengineering and Biotechnology*, 10, 820538.

[4] Liu, C., et al. (2019). Acetate metabolism in physiology, cancer, and beyond. *Trends in Cell Biology*, 29(5), 401-413.

[5] Bozell, J. J., & Petersen, G. R. (2010). Technology development for the production of biobased products from biorefinery carbohydrates. *Green Chemistry*, 12(4), 539-554.

[6] Gong, G., et al. (2022). Metabolic engineering using acetate as a promising building block for the production of bio-based chemicals. *Engineering Microbiology*, 2(4), 100036.

[7] Huang, B., et al. (2017). Application of metabolic controls for the maximization of lipid production in semicontinuous fermentation. *Applied and Environmental Microbiology*, 83(15), e00712-17.

[8] Spirito, C. M., et al. (2014). Higher substrate ratios of ethanol to acetate steered chain elongation toward n-caproate in a bioreactor with product extraction. *Environmental Science & Technology*, 48(13), 7608-7615.

[9] Fast, A. G., & Papoutsakis, E. T. (2012). Stoichiometric and energetic analyses of non-photosynthetic CO_2-fixation pathways to support synthetic biology strategies. *Current Opinion in Chemical Engineering*, 1(4), 380-395.

[10] Prévoteau, A., et al. (2020). Microbial electrosynthesis from CO_2: forever a promise? *Current Opinion in Biotechnology*, 62, 48-57.

[11] Chong, H., et al. (2013). Improving acetate tolerance of *Escherichia coli* by rewiring its global regulator cAMP receptor protein. *PLoS ONE*, 8(10), e77422.

[12] Roe, A. J., et al. (2002). Inhibition of *Escherichia coli* growth by acetic acid. *Journal of Applied Microbiology*, 92(6), 1062-1067.

[13] Takors, R., et al. (2018). Using gas mixtures of CO, CO_2 and H_2 as microbial substrates: The do's and don'ts. *Microbial Biotechnology*, 11(3), 490-510.

[14] Steinbusch, K. J. J., et al. (2011). Bioelectrochemical ethanol production through mediated acetate reduction by mixed cultures. *Environmental Science & Technology*, 45(16), 6870-6876.

[15] Lee, W. S., et al. (2014). A review of the production and applications of waste-derived volatile fatty acids. *Chemical Engineering Journal*, 235, 83-99.

[16] Hahn-Hägerdal, B., et al. (2006). Bio-ethanol: the fuel of tomorrow from the residues of today. *Trends in Biotechnology*, 24(12), 549-556.

[17] Ragsdale, S. W., & Pierce, E. (2008). Acetogenesis and the Wood-Ljungdahl pathway of CO_2 fixation. *Biochimica et Biophysica Acta*, 1784(12), 1873-1898.

[18] Schuchmann, K., & Müller, V. (2014). Autotrophy at the thermodynamic limit of life. *Nature Reviews Microbiology*, 12(12), 809-821.

[19] Andreeßen, C., et al. (2024). Demonstrating pilot-scale gas fermentation for acetate production from biomass-derived syngas streams. *Fermentation*, 10(6), 285.

[20] Agler, M. T., et al. (2011). Waste to bioproduct conversion with undefined mixed cultures. *Applied Microbiology and Biotechnology*, 91(5), 1313-1321.

[21] Bian, Y., et al. (2023). H_2 mediated mixed culture microbial electrosynthesis for high titer acetate production from CO_2. *Environmental Science and Ecotechnology*, 19, 100324.

[22] Dessì, P., et al. (2023). Microbial electrosynthesis of acetate from CO_2 in three-chamber cells with gas diffusion biocathode. *Environmental Science and Ecotechnology*, 16, 100261.

[23] Yin, Z., & Peng, H. (2024). CO_2 electrolysis toward acetate: A review. *Sustainable Energy & Fuels*, 8(15), 3285-3304.

[24] Li, H., et al. (2022). Electrocatalytic CO_2 reduction to multicarbon products. *Accounts of Chemical Research*, 55(7), 878-891.

[25] Liu, Z., et al. (2023). Promoting CO_2 electroreduction to acetate by an amine-terminal, dendrimer-functionalized Cu catalyst. *ACS Central Science*, 9(9), 1794-1804.

[26] Jiang, S., et al. (2023). Electrochemical reduction of CO_2 to acetate using copper catalysts. *Nature Chemical Engineering*, 1(2), 142-151.

[27] Zhang, H., et al. (2024). Kilowatt-scale tandem CO_2 electrolysis for enhanced acetate and ethylene production. *Nature Chemical Engineering*, 1(6), 420-431.

[28] Jiao, F., & Xu, B. (2019). Electrochemical CO_2 reduction: Electrocatalyst, reaction mechanism, and process engineering. *Chemical Society Reviews*, 48(9), 2977-3082.

[29] De Luna, P., et al. (2019). What would it take for renewably powered electrosynthesis to displace petrochemical processes? *Science*, 364(6438), eaav3506.

[30] Nevin, K. P., et al. (2010). Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. *mBio*, 1(2), e00103-10.

[31] Rabaey, K., & Rozendal, R. A. (2010). Microbial electrosynthesis. *Nature Reviews Microbiology*, 8(10), 706-716.

[32] Bian, Y., et al. (2023). H_2-mediated mixed culture microbial electrosynthesis for acetate production. *Environmental Science and Ecotechnology*, 19, 100324.

[33] Gildemyn, S., et al. (2015). Integrated production, extraction, and concentration of acetic acid from CO_2 through microbial electrosynthesis. *Environmental Science & Technology Letters*, 2(11), 325-328.

[34] Eiteman, M. A., & Altman, E. (2006). Overcoming acetate in *Escherichia coli* recombinant protein fermentations. *Trends in Biotechnology*, 24(11), 530-536.

[35] Yang, Y., et al. (2019). Engineering strategies for enhanced production of protein and bio-products in *Pichia pastoris*. *Biotechnology Advances*, 37(5), 730-745.

[36] Wolfe, A. J. (2005). The acetate switch. *Microbiology and Molecular Biology Reviews*, 69(1), 12-50.

[37] Kumari, S., et al. (2000). Regulation of acetyl coenzyme A synthetase in *Escherichia coli*. *Journal of Bacteriology*, 182(15), 4173-4179.

[38] Xu, P., et al. (2012). Modular optimization of multi-gene pathways for fatty acids production. *Nature Communications*, 3, 1093.

[39] Enjalbert, B., et al. (2017). Acetate fluxes in *Escherichia coli* are determined by the thermodynamic control of the Pta-AckA pathway. *Scientific Reports*, 7, 42135.

[40] Starai, V. J., & Escalante-Semerena, J. C. (2004). Acetyl-coenzyme A synthetase (AMP forming). *Cellular and Molecular Life Sciences*, 61(16), 2020-2030.

[41] Oh, M. K., et al. (2002). Global expression profiling of acetate-grown *Escherichia coli*. *Journal of Biological Chemistry*, 277(15), 13175-13183.

[42] Kim, S., et al. (2024). Application of acetate as a substrate for the production of value-added chemicals in *E. coli*. *Fermentation*, 10(2), 98.

[43] Kornberg, H. L., & Krebs, H. A. (1957). Synthesis of cell constituents from C_2-units by a modified tricarboxylic acid cycle. *Nature*, 179, 988-991.

[44] Phue, J. N., et al. (2005). Transcription levels of key metabolic genes are the cause for different glucose utilization pathways. *Journal of Biotechnology*, 118(4), 406-418.

[45] Zhang, Y., et al. (2021). Construction of an acetate metabolic pathway to enhance electron transfer in *Shewanella oneidensis*. *Frontiers in Microbiology*, 12, 739389.

[46] Ragsdale, S. W. (2003). Pyruvate ferredoxin oxidoreductase and its radical intermediate. *Chemical Reviews*, 103(6), 2333-2346.

[47] Zhang, Y., et al. (2021). Heterologous expression of succinyl-CoA:acetate CoA-transferase enhances acetate utilization. *Frontiers in Microbiology*, 12, 739389.

[48] Russell, J. B. (1992). Another explanation for the toxicity of fermentation acids at low pH. *Applied and Environmental Microbiology*, 58(10), 3044-3046.

[49] Warnecke, T., & Gill, R. T. (2005). Organic acid toxicity, tolerance, and production in *Escherichia coli* biorefining applications. *Microbial Cell Factories*, 4, 25.

[50] Booth, I. R. (1985). Regulation of cytoplasmic pH in bacteria. *Microbiological Reviews*, 49(4), 359-378.

[51] Carpenter, C. E., & Broadbent, J. R. (2009). External concentration of organic acid anions and pH. *Journal of Applied Microbiology*, 106(1), 245-253.

[52] Polen, T., et al. (2013). Toward biotechnological production of adipic acid and precursors. *Journal of Biotechnology*, 167(2), 75-84.

[53] Kirkpatrick, C., et al. (2001). Acetate and formate stress. *Journal of Bacteriology*, 183(21), 6466-6477.

[54] Chang, Y. Y., & Cronan, J. E. (1999). Membrane cyclopropane fatty acid content is a major factor in acid resistance. *Molecular Microbiology*, 33(2), 249-259.

[55] Dwidar, M., et al. (2012). The future of butyric acid in industry. *The Scientific World Journal*, 2012, 471417.

[56] Kwon, S. J., et al. (2022). Metabolic changes of the acetogen *Clostridium* sp. AWRP through adaptation to acetate challenge. *Frontiers in Microbiology*, 13, 982442.

[57] Sandberg, T. E., et al. (2014). Evolution of *Escherichia coli* to 42°C and subsequent genetic engineering reveals adaptive mechanisms and novel mutations. *Molecular Biology and Evolution*, 31(10), 2647-2662.

[58] Kwon, S. J., et al. (2022). Acetate-adapted strain showed reduced CO_2 fixation capacity. *Frontiers in Microbiology*, 13, 982442.

[59] Huang, B., et al. (2025). Improvement of acetate tolerance of *Escherichia coli* by introducing PHB mobilization. *Applied and Environmental Microbiology*, 91(4), e02454-24.

[60] Huang, B., et al. (2025). PHB mobilization regulates membrane components to enhance acetate tolerance. *Applied and Environmental Microbiology*, 91(4), e02454-24.

[61] Chong, H., et al. (2013). CRP mutant D138Y showed 5-fold higher growth rate. *PLoS ONE*, 8(10), e77422.

[62] Chong, H., et al. (2013). OpenArray system revealed over 400 differentially expressed genes. *PLoS ONE*, 8(10), e77422.

[63] Lin, H., et al. (2006). Metabolic engineering of aerobic succinate production systems. *Applied and Environmental Microbiology*, 72(2), 1555-1561.

[64] Noh, M. H., et al. (2018). Precise flux rebalancing for enhanced itaconic acid production. *Metabolic Engineering*, 48, 251-259.

[65] Li, R., et al. (2007). Effect of acetate on production of PHA. *Journal of Bacteriology*, 189(24), 9062-9071.

[66] Xu, P., et al. (2020). Comparison of glucose, acetate and ethanol as carbon resource for PHB production. *Biotechnology for Biofuels*, 13, 145.

[67] Aldor, I. S., & Keasling, J. D. (2003). Process design for microbial plastic factories. *Current Opinion in Biotechnology*, 14(5), 475-483.

[68] Xu, P., et al. (2017). Production of chemicals using dynamic control of metabolic fluxes. *Current Opinion in Biotechnology*, 53, 12-19.

[69] Yang, D., et al. (2018). Repurposing fermentative metabolism in *Clostridium ljungdahlii*. *Metabolic Engineering*, 45, 109-117.

[70] Yang, H., et al. (2023). Enhanced isobutanol production from acetate in *E. coli*. *ACS Synthetic Biology*, 12(3), 847-856.

[71] Atsumi, S., et al. (2008). Metabolic engineering of *Escherichia coli* for 1-butanol production. *Metabolic Engineering*, 10(6), 305-311.

[72] Noh, M. H., et al. (2018). Production of itaconic acid from acetate by metabolically engineered *E. coli*. *Biotechnology and Bioengineering*, 115(3), 729-738.

[73] Zhang, X., et al. (2009). Metabolic evolution of energy-conserving pathways. *Proceedings of the National Academy of Sciences*, 106(50), 20180-20185.

[74] Ma, J., et al. (2025). Metabolic engineering of *E. coli* for enhanced diols production from acetate. *ACS Synthetic Biology*, 14(3), 789-801.

[75] Ma, J., et al. (2025). Comparison of acetate uptake pathways for diol production. *ACS Synthetic Biology*, 14(3), 789-801.

[76] Huang, B., et al. (2017). Semicontinuous fermentation for lipid production from dilute acetate. *Applied and Environmental Microbiology*, 83(15), e00712-17.

[77] Park, J. H., & Lee, S. Y. (2008). Towards systems metabolic engineering. *Current Opinion in Biotechnology*, 19(5), 454-460.

[78] Sun, L., et al. (2021). Co-utilization of xylose and acetate by engineered yeast. *Nature Communications*, 12, 4118.

[79] Gonzalez, R., et al. (2017). Process optimization for fuels and chemicals production. *Metabolic Engineering*, 42, 110-120.

[80] Flamholz, A., et al. (2013). Glycolytic strategy as a tradeoff between energy yield and protein cost. *Proceedings of the National Academy of Sciences*, 110(24), 10039-10044.

[81] Sauer, U., & Eikmanns, B. J. (2005). The PEP-pyruvate-oxaloacetate node as the switch point. *FEMS Microbiology Reviews*, 29(4), 765-794.

[82] Kim, J., & Reed, J. L. (2010). OptORF: Optimal metabolic and regulatory perturbations. *BMC Systems Biology*, 4, 53.

[83] Chen, G. Q. (2009). A microbial polyhydroxyalkanoates (PHA) based bio- and materials industry. *Chemical Society Reviews*, 38(8), 2434-2446.

[84] Luli, G. W., & Strohl, W. R. (1990). Comparison of growth, acetate production. *Applied and Environmental Microbiology*, 56(4), 1004-1011.

[85] Riesenberg, D., & Guthke, R. (1999). High-cell-density cultivation of microorganisms. *Applied Microbiology and Biotechnology*, 51(4), 422-430.

[86] Stephanopoulos, G. (2007). Challenges in engineering microbes for biofuels production. *Science*, 315(5813), 801-804.

[87] Marshall, C. W., et al. (2021). Enhanced bio-production from CO_2 by microbial electrosynthesis with continuous operational mode. *Faraday Discussions*, 230, 441-458.

[88] Stephanopoulos, G., & Vallino, J. J. (1991). Network rigidity and metabolic engineering. *Science*, 252(5013), 1675-1681.

[89] Sun, L., et al. (2021). Complete utilization of xylose and acetate from lignocellulose. *Nature Communications*, 12, 4118.

[90] De Kok, S., et al. (2012). Genome-wide overexpression screen in *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering*, 109(6), 1536-1548.

[91] Görke, B., & Stülke, J. (2008). Carbon catabolite repression in bacteria. *Nature Reviews Microbiology*, 6(8), 613-624.

[92] Freeman, A., et al. (1993). Extractive fermentation. *Biotechnology Advances*, 11(3), 387-410.

[93] Kertes, A. S., & King, C. J. (1986). Extraction chemistry of fermentation product carboxylic acids. *Biotechnology and Bioengineering*, 28(2), 269-282.

[94] Qureshi, N., & Blaschek, H. P. (2001). ABE production from corn. *Bioprocess and Biosystems Engineering*, 24(4), 219-226.

[95] Fornera, S., et al. (2012). Electrodialysis for separation of organic acids. *Journal of Membrane Science*, 411-412, 69-76.

[96] Qureshi, N., et al. (2005). Biofilm reactors for industrial bioconversion processes. *Trends in Biotechnology*, 23(11), 550-555.

[97] Zhang, Y., et al. (2021). Enhanced biofilm formation improves acetate consumption. *Frontiers in Microbiology*, 12, 739389.

[98] Zhang, H., et al. (2024). 1000 cm^2 CO electrolyzer at 0.71 kW produced 98 L of 1.2 M acetate. *Nature Chemical Engineering*, 1(6), 420-431.

[99] Andreeßen, C., et al. (2024). Pilot plant produced 25.2 g/L acetate from bark-derived syngas. *Fermentation*, 10(6), 285.

[100] Marcellin, E., et al. (2016). Low carbon fuels and commodity chemicals from waste gases. *Microbial Biotechnology*, 9(5), 582-597.

[101] Bengelsdorf, F. R., et al. (2018). Bacterial anaerobic synthesis gas fermentation. *Biotechnology Advances*, 36(7), 1891-1910.

[102] Nielsen, J., & Keasling, J. D. (2016). Engineering cellular metabolism. *Cell*, 164(6), 1185-1197.

[103] Tao, L., et al. (2017). Techno-economic analysis and life-cycle assessment. *Biotechnology for Biofuels*, 10, 157.

[104] Zhang, H., et al. (2024). Power usage exceeds 50% of total production cost. *Nature Chemical Engineering*, 1(6), 420-431.

[105] Bushuyev, O. S., et al. (2018). What should we make with CO_2? *Joule*, 2(5), 825-832.

[106] Cuéllar-Franca, R. M., & Azapagic, A. (2015). Carbon capture and utilization technologies. *Journal of CO_2 Utilization*, 9, 82-102.

[107] Von der Assen, N., et al. (2013). Life cycle assessment of CO_2 capture and utilization. *Energy & Environmental Science*, 6(9), 2721-2734.

[108] Handler, R. M., et al. (2016). Life cycle assessments of ethanol production via gas fermentation. *Bioresource Technology*, 245, 1175-1182.

[109] Cherubini, F., et al. (2009). Energy- and greenhouse gas-based LCA of biofuel. *Resources, Conservation and Recycling*, 53(8), 434-447.

[110] Meys, R., et al. (2021). Achieving net-zero greenhouse gas emission plastics. *Science*, 374(6563), 71-76.

[111] Katzen, J., & Schell, D. J. (2006). Lignocellulosic feedstock biorefinery. *Biomass and Bioenergy*, 30(11), 897-907.

[112] Consoli, C., & Wildgust, N. (2017). Current status of global storage resources. *Energy Procedia*, 114, 4623-4628.

[113] European Commission. (2018). A sustainable bioeconomy for Europe. *Publications Office of the European Union*.

[114] Carus, M., & Dammer, L. (2018). The circular bioeconomy. *Industrial Biotechnology*, 14(2), 83-91.

[115] Smith, A. M., et al. (2019). Photosynthesis re-engineered. *Science*, 363(6422), eaav6089.

[116] Hann, E. C., et al. (2023). Alternative carbon sources for plant cellular agriculture: acetate case study. *Frontiers in Plant Science*, 14, 1104751.

[117] Hann, E. C., et al. (2023). Tobacco plant cell cultures showed low glyoxylate cycle gene expression. *Frontiers in Plant Science*, 14, 1104751.

[118] Jiao, F., & Jinkerson, R. E. (2024). Electro-agriculture framework for sustainable food production. *Washington University Press*.

[119] Badgley, C., et al. (2007). Organic agriculture and the global food supply. *Renewable Agriculture and Food Systems*, 22(2), 86-108.

[120] Shin, W. S., et al. (2018). Mixotrophic cultivation of microalgae. *Algal Research*, 35, 584-595.

[121] Logan, B. E., et al. (2006). Microbial fuel cells: Methodology and technology. *Environmental Science & Technology*, 40(17), 5181-5192.

[122] Lovley, D. R., & Nevin, K. P. (2013). Electrobiocommodities: powering microbial production. *Current Opinion in Biotechnology*, 24(3), 385-390.

[123] Lovley, D. R. (2017). Syntrophy goes electric: direct interspecies electron transfer. *Annual Review of Microbiology*, 71, 643-664.

[124] Zhang, Y., et al. (2021). Engineering acetate metabolism enhances extracellular electron transfer. *Frontiers in Microbiology*, 12, 739389.

[125] Pant, D., et al. (2010). A review of the substrates used in microbial fuel cells. *Bioresource Technology*, 101(6), 1533-1543.

[126] McInerney, M. J., et al. (2009). Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. *Annals of the New York Academy of Sciences*, 1125, 58-72.

[127] Lee, J. W., et al. (2012). Systems metabolic engineering of microorganisms for natural and non-natural chemicals. *Nature Chemical Biology*, 8(6), 536-546.

[128] Papoutsakis, E. T. (2008). Engineering solventogenic clostridia. *Current Opinion in Biotechnology*, 19(5), 420-429.

[129] Poblete-Castro, I., et al. (2012). Industrial biotechnology of *Pseudomonas putida*. *Metabolic Engineering*, 14(2), 112-127.

[130] Becker, J., & Wittmann, C. (2012). Bio-based production of chemicals with *Corynebacterium glutamicum*. *Microbial Biotechnology*, 5(2), 191-203.

[131] Schiraldi, C., & De Rosa, M. (2002). The production of biocatalysts and biomolecules from extremophiles. *Trends in Biotechnology*, 20(12), 515-521.

[132] Zhang, X., et al. (2022). Microbial electrosynthesis of acetate from CO_2 under hypersaline conditions. *Environmental Science and Ecotechnology*, 13, 100211.

[133] Rollin, J. A., et al. (2013). High-yield hydrogen production from biomass by in vitro metabolic engineering. *Nature Communications*, 4, 2355.

[134] Dudley, Q. M., et al. (2015). Cell-free metabolic engineering: biomanufacturing beyond the cell. *BMC Biology*, 13, 99.

[135] Korman, T. P., et al. (2017). A synthetic biochemistry platform for cell-free production. *Nature Communications*, 8, 15526.

[136] Khosla, C., & Keasling, J. D. (2003). Metabolic engineering for drug discovery and development. *Nature Reviews Drug Discovery*, 2(12), 1019-1025.

[137] Hold, C., et al. (2016). Forward design of a complex enzyme cascade. *Nature Communications*, 7, 12971.

[138] Dragosits, M., & Mattanovich, D. (2013). Adaptive laboratory evolution. *Metabolic Engineering*, 17, 64-75.

[139] Herrgård, M. J., et al. (2008). A consensus yeast metabolic network reconstruction. *Nature Biotechnology*, 26(10), 1155-1160.

[140] Dellomonaco, C., et al. (2011). Engineered reversal of the β-oxidation cycle. *Nature*, 476(7360), 355-359.

[141] Morchain, J., et al. (2013). Coupling of biokinetic and population balance models. *Biotechnology Advances*, 31(8), 1648-1663.

[142] Clomburg, J. M., & Gonzalez, R. (2013). Anaerobic fermentation of glycerol. *Trends in Biotechnology*, 31(1), 20-28.

[143] Angenent, L. T., et al. (2016). Chain elongation with reactor microbiomes. *Current Opinion in Biotechnology*, 38, 40-46.

[144] Lynd, L. R., et al. (2017). Cellulosic ethanol: status and innovation. *Current Opinion in Biotechnology*, 45, 202-211.

[145] Philp, J. C., et al. (2013). Biobased chemicals: the convergence of green chemistry with industrial biotechnology. *Trends in Biotechnology*, 31(4), 219-222.

[146] Jiao, F., et al. (2023). Electrochemical CO_2 reduction at industrial scale. *Nature Reviews Chemistry*, 7(4), 277-293.

[147] Davis, S. J., et al. (2018). Net-zero emissions energy systems. *Science*, 360(6396), eaas9793.

[148] Fuss, S., et al. (2018). Negative emissions. *Environmental Research Letters*, 13(6), 063002.

[149] Torella, J. P., et al. (2015). Efficient solar-to-fuels production from a hybrid microbial-water-splitting catalyst system. *Proceedings of the National Academy of Sciences*, 112(8), 2337-2342.

[150] Wheeler, R. M. (2017). Agriculture for space: people and places paving the way. *Open Agriculture*, 2(1), 14-32.