Qwen

Acetate: A Versatile Feedstock for a Sustainable Biomanufacturing Economy

The Metabolic Architecture of Acetate Assimilation

Acetate stands at a crossroads in modern biotechnology, representing both one of the most promising next-generation feedstocks and a significant metabolic challenge for industrial microorganisms [21 41]. Its fundamental nature as a C2 compound with high water miscibility makes it an attractive substrate for fermentation, yet its weak acid properties confer potent toxicity at elevated concentrations [35 41]. This duality necessitates a deep understanding of its metabolic architecture, from cellular uptake and assimilation to the complex regulatory networks that govern its fate within the cell. The efficiency with which a microbe can convert acetate into acetyl-CoA, the central hub of metabolism, dictates the ultimate success of any bioprocess. This conversion occurs primarily through two distinct enzymatic pathways, each with unique kinetics, energy requirements, and regulatory controls, forming the foundation of acetate utilization strategies [25 63]. Furthermore, for net carbon gain from this highly oxidized molecule, cells must employ specialized bypasses like the glyoxylate shunt, a pathway whose regulation is central to reprogramming metabolism for biosynthesis rather than mere maintenance [23 41].

The assimilation of acetate into central metabolism begins with its transport across the cellular membrane and subsequent activation to acetyl-CoA. Microbes have evolved multiple mechanisms for acetate uptake, including passive diffusion of the undissociated protonated form (CH_3COOH) and active symport systems that couple acetate import with protons or sodium ions [21 23]. Once inside the cytoplasm, where the pH is near neutral (~7.5 – 7.6), the acetate ion (CH_3COO^-) dissociates, releasing a proton and contributing to intracellular acidification—a primary mechanism of its inhibitory action [38 41]. The entry point into metabolism is the formation of acetyl-CoA, catalyzed by two main pathways. The first is the ATP-dependent route mediated by acetyl-CoA synthetase (ACS), also known as acetate thiokinase [25 35]. This enzyme ligates acetate to coenzyme A (CoA) using ATP, producing AMP, pyrophosphate (PPi), and acetyl-CoA [21]. The ACS pathway is characterized by a high affinity for acetate, making it the preferred scavenging system under low-acetate conditions [58 63]. The second major pathway involves a two-step reaction catalyzed by phosphotransacetylase (Pta) and acetate kinase (AckA) [25 63]. First, Pta transfers a phosphoryl group from acetyl-phosphate to ADP to produce ATP and acetate. Then, AckA transfers the acetyl group from acetate to CoA, generating acetyl-CoA and releasing a phosphate group [25]. This Pta-AckA pathway is generally considered lower-affinity and reversible, allowing it to function effectively at higher extracellular acetate concentrations where thermodynamic gradients favor consumption [21 37]. The interplay between these two nodes is a critical control point in cellular metabolism; they compete for the common intermediate acetyl-phosphate and represent a key decision point for whether the cell assimilates or excretes acetate [21 23].

Overexpression of the acs gene is a common metabolic engineering strategy aimed at enhancing acetate uptake and scavenging capabilities [2 3 58].

Once activated to acetyl-CoA, the carbon skeleton enters central metabolism. However, a fundamental energetic limitation arises because acetate is a more reduced compound than glucose, yielding significantly less energy per mole during oxidation [21 63]. While glucose can generate approximately 38 ATP molecules via complete aerobic respiration, acetate yields only about 10 ATP molecules in E. coli after accounting for the 2 ATP consumed to activate it via ACS [21 63]. This lower energy content places a greater reliance on oxidative phosphorylation to meet the cell's energy demands, particularly for biosynthetic processes [63]. To synthesize biomass and value-added products from acetyl-CoA, the cell must perform gluconeogenesis, creating four-carbon intermediates from two molecules of acetyl-CoA. Under normal TCA cycle operation, this process would result in a net loss of two carbon atoms as CO2, rendering it impossible to build biomass from acetate alone [23 63]. To circumvent this, cells employ the glyoxylate shunt, a crucial set of bypass enzymes that allows for the net synthesis of oxaloacetate from acetyl-CoA [41 63]. Key enzymes in this pathway are isocitrate lyase (AceA) and malate synthase (GlcB) [12 19]. By diverting isocitrate away from the decarboxylation steps of the TCA cycle, the glyoxylate shunt enables the cell to accumulate succinate and subsequently oxaloacetate, providing the necessary precursors for amino acid, sugar, and nucleotide biosynthesis [23 41]. Consequently, the glyoxylate shunt is not merely an accessory pathway but a non-negotiable gateway for growth on acetate as a sole carbon source [23]. In E. coli, its expression is tightly regulated by the transcriptional repressor IclR, and deleting iclR is a frequently used engineering strategy to constitutively activate the shunt and enhance carbon flux toward biosynthesis [12 58 62].

The regulation of acetate metabolism extends far beyond the glyoxylate shunt, involving a complex web of global regulators that coordinate the cell's response to carbon availability. Carbon catabolite repression (CCR) is a prime example, whereby the presence of a preferred carbon source like glucose actively suppresses the utilization of alternative substrates, including acetate [38 58]. In E. coli, this is mediated by the cAMP receptor protein (CRP). When glucose is abundant, intracellular cAMP levels are low, preventing CRP from activating transcription of genes required for acetate utilization, most notably the acs gene [38 58]. This creates a characteristic diauxic growth pattern where cells preferentially consume glucose before switching to acetate. Engineering strains with altered CRP activity, for instance by introducing mutations in the CRP gene itself, has been shown to improve acetate tolerance and allow for simultaneous co-utilization of glucose and acetate, thereby overcoming this regulatory barrier [38 75]. Beyond CCR, acetate itself acts as a signaling molecule and a global regulator of central metabolism. Kinetic modeling and transcriptomic analyses have revealed that increasing acetate concentrations lead to widespread downregulation of genes involved in glycolysis and the TCA cycle, indicating an active transcriptional reprogramming that reduces metabolic flux capacity [36]. This suggests that acetate's inhibitory effect is not solely due to physicochemical stress but also involves active metabolic regulation that prioritizes survival over growth under certain conditions [36]. Similarly, in Saccharomyces cerevisiae, the transcription factor Haa1p plays a central role in mediating the acid stress response to acetic acid, highlighting conserved principles of how eukaryotic cells manage acetate stress [76]. Understanding these intricate regulatory networks is paramount for

designing effective metabolic engineering strategies that can override native constraints and direct carbon flux efficiently toward target products.

Engineering Microbial Chassis for Acetate Valorization

The successful transformation of acetate from a metabolic inhibitor into a valuable feedstock hinges on the rational engineering of microbial chassis. A diverse array of organisms, ranging from the model bacterium Escherichia coli to specialized oleaginous yeasts and industrially relevant acetogens, have been metabolically reprogrammed to harness acetate as a primary carbon source for the production of a wide spectrum of biochemicals [21 41]. These engineering efforts typically involve a multi-pronged approach targeting several key areas: enhancing the primary acetate assimilation machinery, redirecting central metabolic flux away from competing pathways and toward the desired product, balancing intracellular redox and energy cofactors, and improving the host's inherent tolerance to high acetate concentrations [41 45]. Each microbial platform possesses unique metabolic characteristics and regulatory networks, necessitating tailored engineering strategies that leverage its specific strengths while mitigating its weaknesses. The progress in this field demonstrates a clear trajectory from basic proof-of-concept demonstrations to sophisticated strain designs capable of achieving high product titers and yields, laying the groundwork for economically viable biomanufacturing processes.

Escherichia coli has emerged as the workhorse of acetate valorization research, benefiting from its well-characterized genetics, rapid growth, and versatile metabolic network [21 34]. Early successes focused on establishing the ability to grow on acetate and produce simple compounds. For instance, engineered strains have successfully produced β-caryophyllene, mevalonate, and succinate from acetate as the sole carbon source [23 34]. More advanced engineering has led to the production of complex molecules like the sweet protein monellin and the recombinant protein MNEI, demonstrating the utility of acetate-based media for industrial protein production [40 58]. The core engineering strategies in E. coli revolve around manipulating the acetyl-CoA node. This often begins with the deletion of competing pathways to prevent carbon diversion. For example, to produce diols from acetate, strains were engineered with deletions of lactate dehydrogenase (ΔldhA), alcohol dehydrogenase (ΔadhE), pyruvate formate-lyase (Δpta), and fumarate reductase (ΔfrdA) to channel carbon flux away from mixed-acid fermentation [43 57]. Simultaneously, the acetate assimilation pathways are enhanced. This commonly involves overexpressing the high-affinity acetyl-CoA synthetase (acs) to improve scavenging [2 3], or overexpressing the citrate synthase (gltA) to increase the flux into the TCA/glyoxylate cycles [12 62]. Crucially, many products require specific precursors or cofactors, prompting further rounds of engineering. To produce isopropanol, a three-carbon molecule, the glyoxylate shunt was activated by deleting its repressor iclR, and redox balance was managed by overexpressing the transhydrogenase pntAB to generate NADPH from NADH [31 57]. Similarly, for lipid production, fatty acid synthesis was deregulated by deleting the key enzyme FadE, freeing up acetyl-CoA for fatty acid biosynthesis [58]. These combinatorial approaches, guided by metabolic modeling, have enabled E. coli to produce a remarkable diversity of chemicals from acetate, including alcohols, terpenoids, amino acids, and bioplastics [21 63].

While E. coli excels at producing a wide variety of chemicals, other microbial platforms have demonstrated superior performance for specific classes of products, particularly lipids and single-cell protein (SCP). Oleaginous yeasts, such as Yarrowia lipolytica and Rhodosporidium toruloides, are naturally adept at accumulating high levels of lipids when grown on various carbon sources, including acetate [25 46]. Yarrowia lipolytica, in particular, has been extensively engineered for lipid production from acetate [8 25]. One study achieved a lipid yield of 0.207 g lipids/g acetate by overexpressing the acylglycerolphosphate acyltransferase gene SLC1, alongside ACC1 and DGA2 [8]. Further improvements were realized by overexpressing GPD and SOL3 to boost NADPH supply, a critical cofactor for fatty acid synthesis [8]. Another strategy involved disrupting mitochondrial transport to increase the accumulation of cytoplasmic acetyl-CoA, the precursor for lipid synthesis [6]. These efforts resulted in lipid titers reaching 46 g/L in fed-batch fermentations, showcasing the immense potential of these yeasts for biofuel and oleochemical production [46 58]. Cryptococcus curvatus has also been successfully used for lipid production from acetate, especially when co-utilized with other sugars from hydrolysates [35 46]. Beyond lipids, oleaginous yeasts are prime candidates for SCP production. Engineered S. cerevisiae strains have been developed to co-utilize acetate with xylose, improving overall carbon recovery from lignocellulosic biomass [35]. A key innovation in yeast engineering involves repurposing acetate as an electron acceptor to alleviate redox imbalance during anaerobic ethanol fermentation. By deleting the glycerol-3-phosphate dehydrogenase genes (Δgpd1 Δgpd2) and expressing a heterologous acetaldehyde dehydrogenase (eadhE or mhpF), the cell can use acetate to reoxidize NADH, thereby reducing costly glycerol formation and increasing ethanol yield [3 7 9]. This clever metabolic rewiring highlights how acetate can be transformed from an inhibitor into a functional co-substrate that improves process efficiency.

Beyond the established workhorses, specialized microbes and emerging technologies are expanding the repertoire of acetate-based biomanufacturing. Natural acetogens, such as Moorella thermoacetica and Clostridium autoethanogenum, are obligate acetate producers from syngas (CO, CO2, H2) and are therefore ideal platforms for gas-to-liquid biorefineries [14 35 72]. These organisms can achieve high acetate titers, with M. thermoacetica producing up to 59.2 g/L in batch fermentation [58]. Their metabolic machinery is centered on the Wood-Ljungdahl pathway, which fixes CO2 or CO into acetyl-CoA, which is then converted to acetate via the Pta-AckA pathway [21 35]. Engineering these organisms focuses on enhancing acetate production and tolerance, or developing synthetic consortia where they serve as the first stage of a two-stage process to convert syngas into acetate, which is then upgraded by a second organism into a higher-value product [21 58]. Another powerful tool for engineering tolerance is adaptive laboratory evolution (ALE). By subjecting fastidious acetate-utilizing strains to prolonged growth under high acetate stress, researchers can select for spontaneous mutations that confer robustness. This approach has been successfully used to improve the growth rate of E. coli on acetate by over 30% and reduce lag phases from hours to minutes [25]. Genome sequencing of evolved strains has identified key mutations in global regulators like CRP and stress-response genes, providing valuable insights for rational engineering [75]. Finally, the development of dynamic regulation systems represents a sophisticated frontier. Instead of static genetic modifications, these systems use biosensors that respond to intracellular metabolites (like acetate) to dynamically modulate gene expression. An acetate-responsive biosensor was used to create a bifunctional circuit that simultaneously represses NAD(P)H-generating TCA cycle genes and

overexpresses an NADH-consuming enzyme (ndh), leading to a 2.04-fold increase in phloroglucinol titer and a dramatic reduction in acetate accumulation [59]. Such systems exemplify the move towards intelligent, responsive metabolic engineering that can optimize production in real-time.

Platform	Target Product(s)	Key Engineering Strategies
Escherichia coli	Succinate, Isopropanol, Lipids, Mevalonate, Tyrosine, Isobutanol, Butyl Acetate	Deletion of competing pathways (ΔsdhAB, ΔldhA, ΔfadE); Overexpression of acs, gltA, iclR; Cofactor engineering (pntAB, nadK); Two-stage fermentation [12 23 31 57 58 60]
Saccharomyces cerevisiae	Ethanol, Ethyl Acetate, Single-Cell Protein	Deletion of GPD1/GPD2; Expression of bacterial eadhE or mhpF; Overexpression of ACS2, ZWF1; Use of acetate as NADH sink [3 6 7 9]
Yarrowia lipolytica	Lipids, Itaconic Acid, Citric Acid	Overexpression of lipid synthesis genes (SLC1, ACC1, DGA2); Disruption of mitochondrial transport; Downregulation of fatty acid synthesis [8 23 25]
Corynebacterium glutamicum	L-Homoserine	Decoupling glycolysis from TCA cycle; Use of acetate as co-substrate with glucose [23 62]
Aspergillus oryzae	Malate	Utilization of high acetate concentrations (45 g/L) as sole carbon source [21]
Clostridium autoethanogenum	Syngas to Acetate Conversion	Native acetogen; Engineered for efficient gas-to-liquid conversion [58 72]

A Panorama of Products and Performance Metrics

The versatility of acetate as a carbon source is reflected in the remarkable breadth of products synthesized by engineered microbes. From bulk commodity chemicals and liquid fuels to high-value pharmaceuticals and nutraceuticals, acetate serves as a foundational building block for a sustainable biomanufacturing economy [21 33]. The performance of these bioprocesses—quantified by metrics such as titer (concentration), yield (grams of product per gram of substrate), and productivity (grams of product per liter per hour)—is a critical determinant of their commercial viability. While laboratory-scale achievements demonstrate the feasibility of converting acetate into a vast array of molecules, translating these successes into economically competitive industrial processes remains a significant challenge, contingent upon continuous improvements in strain performance and process engineering [21 34]. This section provides a comprehensive overview of the products synthesized from acetate, highlighting the highest reported performances and underscoring the current state-of-the-art in acetate-based biomanufacturing.

In the realm of organic acids and related carboxylic esters, acetate has proven to be an excellent precursor. Succinic acid, a key platform chemical for polymers and solvents, has been produced in E. coli from acetate as the sole carbon source. Through extensive metabolic engineering, including deletions of the TCA cycle and malic enzymes, and overexpression of citrate synthase, an engineered E. coli MG1655 strain reached a succinate concentration of 16.5 mM (approximately 1.5 g/L) [12]. Using a resting-cell conversion process with fed-batch feeding, this was further increased to 61.7 mM (about 5.7 g/L) [12]. Other organic acids include malate, produced by Aspergillus oryzae at a titer of 8.44 g/L from 45 g/L acetic acid, and itaconic acid, a promising polymer precursor, produced in Corynebacterium glutamicum at 29.2 g/L using acetate as the sole carbon source [21]. Glycolate, another important chemical intermediate, was produced in E. coli at 73.3 g/L using a dual-module system with acetate and glucose cosubstrates, achieving a record-breaking yield of 1.08 g/g on acetate [21]. Beyond acids, acetate serves as a carbon backbone for flavor and fragrance compounds. Engineered S. cerevisiae has been used to produce ethyl acetate, the primary aroma compound in wine, reaching a titer of 1.69 g/L [6]. In E. coli, isobutyl acetate, a fruity ester, was produced at 19.7 g/L through a clever metabolic design that co-utilized acetate and glucose to circumvent carbon loss [21 58]. Butyl acetate was also produced in E. coli at a high titer of 22.8 g/L in a bench-top bioreactor, representing one of the highest reported titers from a whole-cell system [60].

The production of alcohols and biofuels from acetate showcases the potential for renewable energy carriers. Ethanol, the most common biofuel, has been engineered in S. cerevisiae to be produced from acetate by leveraging it as an electron acceptor to regenerate NADH, which is essential for anaerobic growth and ethanol synthesis [37]. In strains lacking glycerol production pathways, this strategy enabled ethanol yields to increase by 6-13% [39]. Although titers remain modest (e.g., 45 g/L from cellulosic hydrolysate), the demonstration of acetate-to-ethanol conversion is a cornerstone of metabolic engineering for lignocellulosic biorefineries [21]. More reduced, energy-dense alcohols have also been targeted. Isopropanol production in E. coli from acetate was significantly improved through a two-stage process involving nitrogen starvation, which shifted metabolism from growth to product formation [57]. This approach yielded 13.3 g/L isopropanol, the highest titer reported for acetate as the sole carbon source at the time [57]. Isobutanol, a superior gasoline replacement, was produced in shake flasks at 0.1-0.2 g/L, while a more recent study achieved 157.05 mg/L using optimized acetate concentrations [21 31]. Dodecanol, a model long-chain oleochemical, was produced from acetate in E. coli at a theoretical yield of 37%, demonstrating the potential for synthesizing lubricants and surfactants from this feedstock [11]. Even short-chain alcohols like butanol have been produced from acetate, albeit at low titers (~1.4 g/L), highlighting the broader applicability of acetate for fuel production [60].

The synthesis of lipids and bioplastics from acetate is a major focus, given the demand for alternatives to petroleum-derived materials. Oleaginous yeasts like Yarrowia lipolytica and Rhodosporidium toruloides are naturally suited for this task. Y. lipolytica has been engineered to produce triacylglycerides (TAGs), the main component of biodiesel, with a maximum lipid yield of 0.207 g/g acetate [8]. In fed-batch culture, a titer of 46 g/L was achieved, with a productivity of 0.27 g/L/h [46 58]. R. toruloides has also been shown to produce fatty acids from acetate as the sole carbon

source, yielding 2.1 g/L [58]. Polyhydroxyalkanoates (PHAs), a family of biodegradable plastics, have been produced in both E. coli and Pseudomonas putida using acetate as a carbon source [21 63]. In E. coli, various PHA copolymers were produced with yields ranging from 0.218 to 0.358 g/g and productivities between 0.026 and 0.045 g/L/h [21]. P. putida produced 0.21 g/L of mcl-PHA with a yield of 0.03 g/g from acetate [21]. Beyond bulk polymers, acetate is used to produce high-value specialty lipids. Blakeslea trispora was shown to produce β-carotene, a vitamin A precursor, with acetate cosubstrate enhancing its synthesis . Similarly, Rhodotorula glutinis showed enhanced lipid and β-carotene production when cultured with acetate under light exposure [46].

Finally, the production of pharmaceuticals, fine chemicals, and food ingredients from acetate highlights the potential for creating sustainable, bio-based versions of existing commodities. Mevalonate, a key precursor for statins and other drugs, was produced in E. coli at a titer of 7.85 g/L in a two-stage fed-batch process [23 34]. Terpenoids, a class of valuable natural products, have also been synthesized from acetate. β-Caryophyllene, a sesquiterpene with anti-inflammatory properties, was produced in E. coli at 1.05 g/L [21 23]. Aromatic amino acids, such as tyrosine, have been produced from acetate in E. coli at 0.70 g/L, demonstrating the ability to produce bulk amino acids from this feedstock [21 23]. Single-cell protein (SCP), a source of high-quality protein for animal feed and human nutrition, is a particularly compelling application. Coupled fermentation systems using autotrophic bacteria like Clostridium ljungdahlii to produce acetate from industrial off-gas, followed by heterotrophic microbes like Pseudomonas putida or Cupriavidus necator to convert it into biomass, have been techno-economically modeled [10 14]. These processes can produce SCP with crude protein content comparable to soy meal (up to 70%) and a favorable amino acid profile [10 14]. While current yields are still being optimized, the potential to convert waste carbon streams into valuable protein resources is enormous [72]. The table below summarizes the performance of selected acetate-based bioprocesses.

Product	Host Organism	Strategy / Key Features	Titer (g/L)	Yield (g/g)	Productivity (g/L/h)
Isopropanol	E. coli W	Two-stage fermentation, nitrogen starvation	13.3 ± 0.7	0.235 ± 0.030	Not Available [57]
Lipids (TAGs)	Yarrowia lipolytica	Overexpression of SLC1, ACC1, DGA2	46.0	0.207	0.27 [8 46]
Isobutanol	E. coli WY002	Optimized acetate concentration (50 mM)	0.157 ± 0.008	Not Available	Not Available [31]
Itaconic Acid	Corynebacterium glutamicum	Sole carbon source, pH/DO-coupled feeding	29.2	0.21	0.63 [21]

Product	Host Organism	Strategy / Key Features	Titer (g/L)	Yield (g/g)	Productivity (g/L/h)
Mevalonate	E. coli BL21(DE3)	Two-stage fed-batch	7.85	0.27	0.13[23 34]
Succinate	E. coli (resting cells)	Fed-batch with acetate feeding	5.7	0.46	Not Available[12]
Butyl Acetate	E. coli YA5	Bench-top bioreactor, in situ stripping	22.8 ± 1.8	0.12	~0.28 (first 72h)[60]
Single-Cell Protein	Cupriavidus necator	Gas fermentation (from CO), coupled process	>14.0 (biomass conc.)	Up to 0.70 (protein)	2.0 - 3.0[14]
Ethyl Acetate	Saccharomyces cerevisiae PGAeΔPOR2	Co-expression of ATF1, ACS1/2, ALD6	1.69	Not Available	Not Available[6]

Techno-Economic and Life Cycle Analysis of Acetate-Based Processes

The transition of acetate-based biomanufacturing from laboratory innovation to industrial reality is critically dependent on its economic competitiveness and environmental sustainability. Techno-economic analysis (TEA) and life cycle assessment (LCA) provide the quantitative frameworks necessary to evaluate these dimensions, revealing the key drivers of cost, the major sources of environmental impact, and the strategic levers for improvement [10 11]. The TEA of acetate-based processes consistently demonstrates that productivity, titer, and yield are the primary determinants of profitability, directly influencing the minimum selling price (MSP) of the final product [10 11]. Conversely, LCAs reveal that the environmental footprint is profoundly sensitive to upstream energy inputs, the choice of purification technology, and the management of co-products [15 16]. A comprehensive analysis shows that while acetate offers a sustainable alternative to fossil-derived feedstocks, its economic viability often requires significant process intensification and optimization of ancillary operations to overcome the inherent energetic limitations of acetate metabolism [21 66].

TEAs of acetate-based bioprocesses consistently identify high volumetric productivity, high cell density, and high product yield as the most impactful factors for reducing the MSP [10]. In a techno-economic model for producing single-cell protein (SCP) from steel mill off-gas via a coupled fermentation process, the estimated MSP ranges from €1.5 to €2.5 per kg of dry matter [10]. This figure is highly sensitive to operational parameters; for instance, achieving an acetate concentration of 45 g/L and a productivity of 4.0 g/L/h in the initial gas-to-acetate fermentation could reduce the unit production cost by 33% to $2.78/kg, largely by decreasing the capital expenditure required for bioreactors [14]. Similarly, a TEA for producing dodecanol from acetate concluded that the

oleochemical yield is the primary driver of both the MSP and the carbon intensity, with scenarios showing that yields exceeding 37% of the theoretical maximum could make the process cheaper and more sustainable than fossil fuel-based routes [11]. Operational expenditure (OPEX) is dominated by energy inputs and downstream processing, which can account for a substantial portion of total costs [10 14]. In a bio-acetic acid plant, downstream separation and purification accounted for over 60% of the total production cost, highlighting the need for energy-efficient recovery methods [66]. The choice of energy source is another dominant economic driver. In the same bio-acetic acid case, natural gas prices were found to be a major cost factor, with a doubling of the price potentially increasing the MSP by over 50% [66]. This underscores the vulnerability of these processes to fluctuations in fossil fuel markets and reinforces the strategic importance of integrating with renewable energy sources.

The environmental performance of acetate-based processes, as evaluated by LCA, presents a complex picture where the benefits of using a renewable, non-food-competing feedstock can be offset by the energy-intensive nature of some production and purification steps. A comparative LCA of bio-acetic acid production from poplar biomass versus petroleum-based methanol carbonylation revealed that the bio-based route can achieve a significantly lower Global Warming Potential (GWP) and Fossil Fuel Use (FFU) [15 16 64]. Specifically, the bio-acetic acid process using alamine/diisobutyl ketone (DIBK) solvent extraction achieved a GWP of -370 to 180 kg CO2-eq/tonne, meaning it can be carbon-negative, whereas the petroleum-based process has a GWP of +1000 kg CO2-eq/tonne [15 16]. The negative GWP is largely attributed to the sequestration of atmospheric carbon from biomass and the use of avoided production credits from displacing natural gas-based electricity when lignin co-products are burned onsite [15 16]. However, the choice of downstream purification method is critical. An alternative process using ethyl acetate extraction resulted in a much higher GWP of 1000 − 2500 kg CO2-eq/tonne, primarily due to the higher energy demand for distillation [15 16]. Similarly, the management of lignin co-products significantly impacts the environmental balance. Exporting lignin for co-firing at a coal power plant results in higher GWP and FFU compared to burning it onsite for energy generation, as the latter displace fossil fuel combustion [15 64]. These findings highlight that a holistic, system-level approach is necessary for LCA, where decisions regarding energy sourcing, co-product valorization, and process integration have profound effects on the overall sustainability of the bioprocess.

The interplay between economic and environmental performance reveals a central tension in the design of acetate-based biorefineries. On one hand, maximizing energy efficiency and minimizing process complexity can improve economic viability. For example, microbial electrosynthesis (MES) for acetate production from CO2 is touted for its high potential for sustainability, but its current low space-time yield means it still has a higher environmental impact than fossil routes unless powered by 100% renewable energy [65]. On the other hand, achieving economic competitiveness often relies on economies of scale and the use of mature, albeit energy-intensive, technologies like distillation [13 66]. The capital cost for a cellulosic biorefinery is estimated to be about 3.5 times higher per unit of capacity than a traditional methanol carbonylation plant, reflecting the complexity of the bioconversion equipment [66]. Therefore, a key challenge lies in bridging this gap by developing more efficient, scalable, and lower-cost technologies for both upstream acetate production and downstream product recovery. Process intensification, such as the targeted increase in acetate productivity in the gas-to-acetate fermentation step mentioned earlier, offers a clear path to reducing

both capital and operating costs [14]. Furthermore, integrating product recovery with the bioprocess itself, for example, through in-situ removal of volatile products like isopropanol or butyl acetate, can alleviate product toxicity, improve yields, and reduce downstream processing costs [60 63]. Ultimately, the future economic and environmental success of acetate biomanufacturing will depend on a synergistic approach that combines advanced metabolic engineering with innovative process design, energy management, and a commitment to circularity throughout the entire value chain.

The Cell-Free Frontier and Emerging Production Paradigms

While whole-cell microbial fermentation remains the dominant paradigm for biomanufacturing, the emergence of cell-free systems represents a disruptive technological shift with the potential to overcome many of the inherent limitations of living cells [22 26]. These systems consist of purified enzymes, cofactors, and other essential components assembled in vitro to reconstruct metabolic pathways for the synthesis of target chemicals [26 28]. By operating outside the context of a cell, these artificial biosystems offer unparalleled control over reaction conditions, eliminate constraints imposed by cellular viability and gene regulation, and exhibit superior tolerance to inhibitory substrates and products [22 26]. This makes them particularly well-suited for producing toxic or complex molecules that are challenging to synthesize in vivo. Recent advancements in enzyme engineering, cofactor regeneration, and system design are rapidly expanding the capabilities of cell-free platforms, positioning them as a powerful complementary technology to microbial fermentation and paving the way for a new generation of sustainable chemical production.

The primary advantage of cell-free systems lies in their ability to operate independently of cellular physiology. Without membranes to restrict mass transfer or pumps to expend energy, these systems can achieve higher reaction rates and better access to substrates [27 28]. They can be operated under non-physiological conditions, such as extreme pH or temperature, which can enhance reaction kinetics or enable chemistries inaccessible to living organisms [26 28]. For instance, performing reactions at low pH (<5) can increase the reduction potential of carboxylic acids, potentially allowing for direct NAD(P)H-dependent reduction without the ATP-consuming activation step required in vivo [26 28]. This flexibility is exemplified by the construction of a cell-free pathway for fumarate production from acetate and glyoxylate, which utilized three purified enzymes from E. coli (Acs, GlcB, and FumC) and achieved a 34% conversion rate from 1 mM acetate [19 20]. Another landmark achievement was the development of a completely in vitro system for phloroglucinol production from acetate, which used enzymes from Acetobacter pasteurianus and Pseudomonas fluorescens to reach a yield of 0.64 g/g acetic acid, a feat impossible to achieve in a living cell due to product toxicity [22]. Furthermore, cell-free systems avoid the problem of metabolic crosstalk, where engineered pathways interfere with the host's native metabolism, and eliminate the need to manage competing pathways that drain carbon and cofactors away from the target product [26 27].

Despite their promise, the widespread adoption of cell-free systems is hindered by significant practical challenges, chief among them being the high cost and instability of purified enzymes and cofactors [22 26]. The constant turnover of expensive catalysts and the degradation of labile cofactors like ATP and NAD(P)H over time limit the operational lifetime and economic feasibility of these

systems [22,28]. To address this, researchers have developed sophisticated cofactor regeneration modules. For ATP, systems based on polyphosphate kinase (PPK) and inorganic polyphosphate (polyPn) have been integrated to continuously recycle ADP back to ATP, drastically reducing the input requirement [19,26]. For NAD(P)H, regeneration can be achieved using sacrificial substrates like formate or phosphite, which are oxidized to drive the reduction of NAD(P)+ [26]. Dynamic homeostasis strategies, such as a 'glycolytic rheostat' combining different forms of glyceraldehyde-3-phosphate dehydrogenase, have been designed to balance NADPH consumption and regeneration in real-time, enabling high-yield isobutanol production in a cell-free system [26]. To combat enzyme instability, strategies like immobilization on nanoflowers or encapsulation in protective vesicles are being explored to enhance thermal stability and allow for reuse, with one such system retaining 80% of its activity after 30 catalytic cycles [27]. The development of more stable enzyme analogues and non-canonical redox cofactors (NRCs) with enhanced stability and tunable properties is another active area of research, promising to further lower costs and expand the operational window of cell-free systems [27].

Looking forward, the future of acetate-based production may lie in hybrid approaches that combine the strengths of both cell-free and whole-cell systems [26,28]. One promising strategy involves using crude cell lysates from engineered E. coli as a low-cost source of enzymes and cofactors, which can then be combined with purified enzymes for a specific part of the pathway [26]. This modular approach allows for rapid prototyping and screening of different enzyme variants and concentrations without the need for full genetic re-engineering, accelerating the design-build-test cycle [26,28]. For example, a crude extract from E. coli engineered to express three heterologous enzymes achieved a 2,3-butanediol titer of over 80 g/L with a productivity of 11 g/L/h, rivaling the performance of whole-cell fermentations [26]. Another hybrid concept integrates cell-free substrate degradation with microbial fermentation in a single reactor, creating a seamless bioprocessing pipeline [26]. The ultimate vision for cell-free systems is the assembly of fully autonomous, self-regulating biosynthetic factories. The CETCH cycle, a synthetic pathway for CO2 fixation, is a testament to this ambition, consisting of 17 enzymes from nine different organisms, a polyphosphate-driven ATP regeneration system, and even a proofreading enzyme to correct errors, all assembled and operating in a test tube [26,28]. As the cost of enzymes decreases and the reliability of these systems increases, cell-free platforms are poised to play a transformative role, particularly in the production of high-value pharmaceuticals and fine chemicals where purity and specificity are paramount, and in processes requiring harsh reaction conditions that are incompatible with living cells.

Future Perspectives: Integration, Policy, and the Circular Bioeconomy

The future trajectory of acetate biomanufacturing over the next five to ten years will be defined by a confluence of scientific innovation, strategic investment, and a growing imperative to address climate change and resource scarcity. The current momentum, driven by breakthroughs in metabolic engineering and synthetic biology, is now being amplified by strong national policies and large-scale public-private partnerships dedicated to advancing a circular bioeconomy [47,48,49]. The overarching goal

is to establish a sustainable manufacturing ecosystem where acetate serves as a versatile intermediary, connecting waste carbon streams to a wide array of high-value products, thereby decoupling industrial production from fossil fuels and arable land [11 42]. Realizing this vision will require continued progress in several key areas, including the development of integrated, multi-stage biorefineries, the resolution of regulatory hurdles for novel food and feed products, and the advancement of long-term stability for emerging platforms like cell-free systems.

One of the most significant trends shaping the future of the field is the push toward integrated, multi-stage biorefineries that maximize carbon efficiency and valorize waste streams. The concept of a "chem-bio hybrid" system, where electrochemical or biological processes first convert CO2 or industrial off-gases into soluble liquid intermediates like acetate, followed by microbial or enzymatic upgrading to final products, is gaining traction [42 49]. Initiatives like the CURB (Carbon Utilization Redesign for Biomanufacturing-Empowered Decarbonization) Engineering Research Center are explicitly focused on developing such systems, aiming to create a 10-times more efficient process than natural photosynthesis [49]. These integrated approaches are particularly powerful because they can utilize a diverse range of waste carbon sources, including the CO-rich off-gases from steel mills, cement plants, and ammonia production facilities [10 18 72]. For example, Beijing Shougang Technology has already industrialized a process to produce single-cell protein from CO-rich off-gas using Clostridium autoethanogenum, demonstrating the immediate commercial potential of this strategy [72]. The future will likely see the coupling of gas fermentation with engineered acetate-utilizing hosts like Yarrowia lipolytica for lipid production or E. coli for organic acids, creating closed-loop systems where waste emissions become a primary feedstock [42 58]. This approach not only addresses the economic challenge of feedstock cost but also delivers significant environmental co-benefits, such as reduced greenhouse gas emissions and mitigation of industrial pollution [10 14].

To accelerate the transition to a bio-based economy, governments worldwide are implementing supportive policies and committing substantial funding. In the United States, the National Biotechnology and Biomanufacturing Initiative, launched by the Biden Administration, aims to coordinate federal efforts to advance biotechnology for climate, health, and economic goals [48]. The U.S. Department of Energy is investing hundreds of millions of dollars in R&D for next-generation biofuels and bioproducts, with a specific focus on scaling up promising biotechnologies and expanding domestic biomanufacturing capacity [47]. Large-scale NSF-funded centers, such as CURB, bring together academia, industry, and government to tackle grand challenges in carbon utilization, fostering innovation in fields like electrochemistry and synthetic biology [49 50]. These investments are crucial for de-risking early-stage technologies and building the infrastructure needed to support a growing bioeconomy. Policy instruments like carbon pricing and green subsidies will also play a vital role in leveling the playing field for bio-based products, making them more competitive against incumbent fossil-based counterparts [10]. The alignment of scientific progress with strategic policy support creates a powerful feedback loop, driving both innovation and market adoption.

Despite the immense promise of acetate-based biomanufacturing, several critical knowledge gaps and challenges must be addressed for widespread adoption. First, there is a need for a deeper mechanistic understanding of acetate's dual role as both an inhibitor and a beneficial co-substrate. Unraveling the molecular signals and regulatory networks that determine its metabolic fate under different

physiological conditions is essential for designing more robust and predictable strains [36 39]. Second, the long-term stability of cell-free systems remains a major bottleneck. Developing robust enzyme stabilization techniques, efficient cofactor recycling cycles, and scalable reactor designs is critical for moving these systems from the laboratory to industrial application [22 26]. Third, for applications in food and feed, navigating the complex regulatory landscape is paramount. Establishing clear guidelines and conducting rigorous safety assessments for novel SCP and food ingredients derived from acetate-utilizing microbes—including evaluations of allergenicity, nucleic acid content, and potential toxins—are necessary for consumer acceptance and market access [67 69 71]. Finally, there is a pressing need for more comprehensive genome-scale metabolic models specifically parameterized for acetate utilization. Such models would enable predictive simulations of strain behavior, guiding rational engineering efforts and accelerating the development of high-performance cell factories [34 62]. In conclusion, the next decade will be pivotal for acetate biomanufacturing. With continued scientific breakthroughs, strategic investment, and a clear vision for a circular bioeconomy, acetate is poised to become a cornerstone of a sustainable industrial future, transforming waste into wealth and helping to secure a more resilient and environmentally sound world.

Reference

1. Acetate Metabolism and the Inhibition of Bacterial Growth by ... https://pmc.ncbi.nlm.nih.gov/articles/PMC6560135/
2. Metabolic Engineering of Saccharomyces cerevisiae for ... https://www.mdpi.com/2311-5637/9/11/984
3. Increasing Anaerobic Acetate Consumption and Ethanol ... https://journals.asm.org/doi/10.1128/AEM.01689-15
4. Metabolic engineering strategies for optimizing acetate ... https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-017-0791-3
5. Advanced metabolic Engineering strategies for the ... https://pmc.ncbi.nlm.nih.gov/articles/PMC11681767/
6. Metabolic Engineering of Saccharomyces cerevisiae for Ethyl ... https://pubs.acs.org/doi/abs/10.1021/acssynbio.0c00446
7. Optimizing the balance between heterologous acetate https://pmc.ncbi.nlm.nih.gov/articles/PMC10647013/
8. Metabolic engineering strategies for increasing lipid ... https://dspace.mit.edu/handle/1721.1/103274
9. Pathway engineering strategies for improved product yield ... https://pure.tudelft.nl/ws/portalfiles/portal/110850277/1_s2.0_S2405805X21000995_main.pdf
10. Single-Cell Protein Production from Industrial Off-Gas ... https://www.mdpi.com/2311-5637/9/8/771

11. Demonstration and technoeconomic analysis of dodecanol ... https://pubmed.ncbi.nlm.nih.gov/41161633/
12. Demonstration and technoeconomic analysis of dodecanol ... https://www.researchgate.net/publication/396970772_Demonstration_and_technoeconomic_analysis_of_dodecanol_production_from_acetate_using_metabolically_engineered_Escherichia_coli
13. Techno - economic analysis of the production of acetic acid via ... https://scijournals.onlinelibrary.wiley.com/doi/abs/10.1002/bbb.2299
14. Techno-Economic Analysis for a Coupled Fermentation Appr https://pdfs.semanticscholar.org/4749/6f963a59a81d9f1c3e9c59d0ae707a20cc57.pdf
15. Production routes to bio-acetic acid: life cycle assessment https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-020-01784-y
16. (PDF) Production routes to bio-acetic acid: life cycle ... https://www.researchgate.net/publication/344073191_Production_routes_to_bio-acetic_acid_life_cycle_assessment
17. Life Cycle Assessment of Drop-in Bio-jet Fuel and Acetic Acid ... https://digital.lib.washington.edu/researchworks/items/2d51bda7-2302-44c0-bd4b-e3970d11cf2a
18. Industrial Off-Gas Fermentation for Acetic Acid Production https://www.mdpi.com/2311-5629/11/3/54
19. Conversion of acetate and glyoxylate to fumarate by a cell-free ... https://pmc.ncbi.nlm.nih.gov/articles/PMC10033897/
20. Conversion of acetate and glyoxylate to fumarate by a cell- ... https://www.sciencedirect.com/science/article/pii/S2405805X23000224
21. Metabolic engineering using acetate as a promising building ... https://pmc.ncbi.nlm.nih.gov/articles/PMC11610983/
22. An in vitro synthetic biosystem based on acetate for production ... https://bmcbiotechnol.biomedcentral.com/articles/10.1186/s12896-017-0376-z
23. Acetate as a potential feedstock for the production of value- ... https://www.researchgate.net/publication/350432172_Acetate_as_a_potential_feedstock_for_the_production_of_value-added_chemicals_Metabolism_and_applications
24. Ranking economic and environmental performance of ... https://www.sciencedirect.com/science/article/pii/S2590262825000048
25. Microbial Utilization of Next-Generation Feedstocks for the ... https://pmc.ncbi.nlm.nih.gov/articles/PMC9035589/
26. A critical comparison of cellular and cell-free bioproduction ... https://pmc.ncbi.nlm.nih.gov/articles/PMC7610769/

27. Table 1 Comparison of microbial and cell-free systems for... https://www.researchgate.net/figure/Comparison-of-microbial-and-cell-free-systems-for-biomanufacturing-grouped-by_tbl1_255771115

28. A critical comparison of cellular and cell-free bioproduction ... https://www.sciencedirect.com/science/article/pii/S0958166918301861

29. Breaking the bounds of biology: innovations in cell-free ... https://www.tierrabiosciences.com/blog/innovations-in-cell-free-protein-synthesis

30. What Is the Difference Between Cell-Free and Cell-Based ... https://synapse.patsnap.com/article/what-is-the-difference-between-cell-free-and-cell-based-expression-systems

31. Synthesis of isobutanol using acetate as sole carbon source in ... https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-023-02197-w

32. Significance of Acetate as a Microbial Carbon and Energy ... https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2018GB006129

33. Towards biobased industry: acetate as a promising feedstock ... https://academic.oup.com/femsle/article/365/20/fny226/5101431

34. Fed-batch fermentation using acetate as the sole carbon ... https://www.researchgate.net/figure/Fed-batch-fermentation-using-acetate-as-the-sole-carbon-source-Induction-was-performed_fig3_317326078

35. From Acetate to Bio-Based Products: Underexploited ... https://www.sciencedirect.com/science/article/abs/pii/S0167779920302419

36. Control and regulation of acetate overflow in Escherichia coli https://elifesciences.org/articles/63661

37. Overflow metabolism in bacterial, yeast, and mammalian ... https://www.embopress.org/doi/10.1038/s44320-025-00145-x

38. Acetate fluxes in Escherichia coli are determined by the ... https://www.nature.com/articles/srep42135

39. Acetate is a beneficial nutrient for E. coli at low glycolytic flux https://pmc.ncbi.nlm.nih.gov/articles/PMC10390867/

40. Acetate: friend or foe? Efficient production of a sweet protein in ... https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-015-0299-0

41. Recent advances and challenges in the bioconversion of ... https://www.sciencedirect.com/science/article/abs/pii/S0960852422013979

42. Acetate as a Platform for Carbon-Negative Production ... https://arpa-e.energy.gov/programs-and-initiatives/search-all-projects/acetate-platform-carbon-negative-production-renewable-fuels-and-chemicals

43. From Acetate to Bio-Based Products: Underexploited ... https://www.researchgate.net/publication/346020797_From_Acetate_to_Bio-Based_Products_Underexploited_Potential_for_Industrial_Biotechnology

44. NETL Innovation Efficiently Converts CO2 Into Acetate for ... https://netl.doe.gov/node/12678

45. Recent advances and challenges in the bioconversion of ... https://www.researchgate.net/publication/364101717_Recent_advances_and_challenges_in_the_bioconversion_of_acetate_to_value-added_chemicals

46. Valorization of waste streams and C1 gases for sustainable ... https://www.sciencedirect.com/science/article/abs/pii/S0048969723034186

47. FACT SHEET: The U.S. Government Funds Biotechnology ... https://www.energy.gov/eere/bioenergy/articles/fact-sheet-us-government-funds-biotechnology-and-biomanufacturing

48. Executive Order on Advancing Biotechnology and ... https://bidenwhitehouse.archives.gov/briefing-room/presidential-actions/2022/09/12/executive-order-on-advancing-biotechnology-and-biomanufacturing-innovation-for-a-sustainable-safe-and-secure-american-bioeconomy/

49. WashU to lead $26 million decarbonization initiative https://source.washu.edu/2024/08/washu-to-lead-26-million-decarbonization-initiative/

50. Texas A&M joins $26 million initiative to revolutionize ... https://www.eurekalert.org/news-releases/1057335

51. Acetic Acid Bacteria in the Food Industry - PubMed Central https://pmc.ncbi.nlm.nih.gov/articles/PMC6117990/

52. 1.6: Vinegar and Acetic Acid Fermentation https://chem.libretexts.org/Bookshelves/Biological_Chemistry/Fermentation_in_Food_Chemistry_(Graham)/01%3A_Modules/1.06%3A_Acetic_Acid_Fermentation

53. Acetic acid bacteria https://en.wikipedia.org/wiki/Acetic_acid_bacteria

54. Role of Acetic Acid Bacteria in Food and Beverages https://www.ftb.com.hr/archives/1797-role-of-acetic-acid-bacteria-in-food-and-beverages

55. Acetic acid bacteria are involved in various foods, ... https://www.researchgate.net/figure/Acetic-acid-bacteria-are-involved-in-various-foods-beverages-chemicals-and_fig1_365441427

56. Rediscovering Acetate Metabolism: Its Potential Sources and ... https://pubs.acs.org/doi/abs/10.1021/acs.jafc.8b00458

57. Efficiency of acetate-based isopropanol synthesis in ... https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-024-02534-0

58. (PDF) Towards biobased industry: Acetate as a promising ... https://www.researchgate.net/publication/327822688_Towards_biobased_industry_Acetate_as_a_promising_feedstock_to_enhance_the_potential_of_microbial_cell_factories

59. Engineering an Overflow - Responsive Regulation System for ... https://pmc.ncbi.nlm.nih.gov/articles/PMC12067040/

60. Metabolic engineering of Escherichia coli for efficient ... https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-022-01755-y

61. High-Yield Biosynthesis of 3-Hydroxypropionic Acid from ... https://pubs.acs.org/doi/abs/10.1021/acssynbio.5c00030

62. Use of acetate as substrate for sustainable production ... https://www.sciencedirect.com/science/article/abs/pii/S1096717624000703

63. Microbial Upgrading of Acetate into Value-Added Products ... https://www.mdpi.com/1422-0067/21/22/8777

64. Production routes to bio-acetic acid: life cycle assessment https://pubmed.ncbi.nlm.nih.gov/32905422/

65. is it sustainable for bioproduction of acetic acid? https://pubs.rsc.org/en/content/articlehtml/2021/ra/d1ra00920f

66. Techno-Economic Analysis of Producing Glacial Acetic ... https://www.mdpi.com/1420-3049/25/18/4328

67. EFSA Clarifies Data Requirements for Microorganisms ... https://www.food-safety.com/articles/10851-efsa-clarifies-data-requirements-for-microorganisms-used-in-food-chain

68. Safety and regulatory frameworks for unconventional food ... https://www.sciencedirect.com/science/article/pii/B9780443333453000389

69. Safety of Alternative Proteins: Technological ... https://pmc.ncbi.nlm.nih.gov/articles/PMC8230205/

70. Microorganisms & Microbial-Derived Ingredients Used in ... https://www.fda.gov/food/generally-recognized-safe-gras/microorganisms-microbial-derived-ingredients-used-food-partial-list

71. Addressing the safety of new food sources and production ... https://ift.onlinelibrary.wiley.com/doi/10.1111/1541-4337.13341

72. Metabolic engineering for single-cell protein production ... https://link.springer.com/article/10.1007/s44307-024-00042-8

73. butyl acetate - cfsanappsexternal.fda.gov https://hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=FoodSubstances&id=BUTYLACETATE

74. GRAS Flavoring Substances 18 https://www.femaflavor.org/sites/default/files/18.%20GRAS%20Substances%20%283816-3905%29.pdf

75. Improving Acetate Tolerance of Escherichia coli by Rewiring ... https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0077422

76. Identification of an acetate-tolerant strain ... https://www.sciencedirect.com/science/article/abs/pii/S1389172312002848