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Engineered Microbial Carbon-Capture Pathways for Sustainable Biomanufacturing of Bioplastics

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ABSTRACT

The global environmental crisis provoked by plastics made of petroleum products demonstrates that the alternatives must be sustainable and biodegradable. Microbial fermentation is a promising source of bioplastics, though more established technology uses sugar- and biomass-derived feedstocks which compete with agriculture and increase the cost of production. This article aims to evaluate how bioplastic production can be revolutionized by using engineered microbial carbon-capture pathways to use CO_2 and industrial off-gases as major sources of carbons. The review methodologically summarizes the current progress in metabolic engineering and synthetic biology, discussing natural biosynthetic pathways like the CalvinBensonBassham and the WoodLjungdahl cycles, synthetic pathways like the CETCH cycle or the reductive glycine pathway. The efficiency of carbon fixation is compared, along with the integration of the pathways into microbial hosts, and connection with subsequent bioplastic biosynthesis, i.e. polyhydroxyalkanoates (PHAs) and polylactic acid (PLA). The outcomes of the latest experimental and pilot-scale research indicate that the engineered strains have the potential of reaching as high as 30 percent cost reduction and 50-60 percent lower CO 2 emission, to the production of bioplastics, as compared to the conventional bioplastic production. Moreover, the analysis of techno-economic and lifecycle shows the possibility of climate-positive production in case of incorporating renewable energy sources. The conclusions highlight both opportunities and challenges: on the one hand, improvements in pathway efficiency and bioreactor design is driving progress faster, on the other hand, there are still problems with enzyme kinetics, energy requirements, and scale implementations. This review places microbial carbon-capture biomanufacturing as a disruptive platform in the bioeconomy of the U.S. and globally, and a promising pathway to sustainable, circular, and low-carbon plastic replacement.

1. INTRODUCTION

Plastic pollution has become one of the most urgent environmental issues and since its production is more than 400 million tons a year, a large part of it remains unrecycled and is stored in landfills and the oceans without breaking down. Traditional plastics are mainly made of fossil fuels and besides being a major source of greenhouse gases, this type of plastic aggravates climatic change and disturbance of the ecosystems [1]. Bio plastics like polyhydroxyalkanoates (PHAs) and polylactic acid (PLA) have been suggested as biodegradable. Nevertheless, their massive usage is limited because of the expenses of production and dependence on sugar or starch feedstocks, which are competing with food stocks and farmland [2]. Recent promotion of synthetic biology and metabolic engineering is a disruptive strategy to the production of sustainable polymers. Programmed microbial systems with the ability to capture and fix carbon provide the possibility of turning CO 2 and industrial gas directly into precursors of bioplastics. Carbon assimilation can be based on natural pathways, including the Calvin194120121 Benson194120121 Bassham 194120121Bassham194120121Bassham1941201 21Bassham194120121Bassham194120121Bassha m194120121Bassham194120121Bassham1941

Artificial variants, such as the crotonyl-CoA/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle, and artificial reductive glycine pathway have been shown to have better energy efficiency and carbon conversion capacity [3], [4]. In spite of the positive news, the available literature does not offer detailed approaches that can bridge carbon capture with bioplastic production platforms that can be scaled to the industrial level. A majority of the studies are still at the laboratory level with little extending to technological economic and lifecycle sustainability evaluation. Further, the rate of enzymes, cofactor interactions, and energy requirements are bottlenecks, which limit the efficiency of the pathways [5].

This review fills these gaps through a systematic review of carbon-capture pathways of microbes in producing bioplastics. The article (i) assesses both natural and synthetic pathways to CO 2 assimilation, (ii) links pathway engineering to the production of PHAs and PLA, (iii) compares the performance measures of recent experimental and pilot studies, and (iv) explains future perspectives on integrating microbial carbon-capture systems into a circular bioeconomy.

2. RELATED WORK

2.1 Microbial Bioplastic Production

Conventional microbial fermentation pathways to polyhydroxyalkanoates (PHAs) and polylactic acid (PLA) have been based on sugar- and starch-derived substrates. Organisms, including Cupriavidusnecator and recombinant strains of Escherichia coli have been actively optimized towards the accumulation of PHAs under nutrient-limiting conditions [6]. Though these routes show high product titers, their reliance on agricultural feedstocks poses sustainability issues and makes the process of production very costly [7].

2.2 Carbon Fixation in Microbes

Naturally, autotrophic microorganisms such as cyanobacteria and hydrogen-oxidizing bacteria take up CO 2 by the CalvinBensonBassham (CBB) cycle or by the WoodLjungdahl pathway [8]. These systems are directly utilized in the form of CO 2 usage, eliminating the use of external organic substrates. Nevertheless, their intrinsic shortcomings, such as poor catalytic efficiency of RuBisCO, slow growth rates and exposure to environmental changes, limit their potential use in industry [9].

2.3 Synthetic Biology Approaches

Synthetic biology has made it possible to engineer heterotrophic hosts, including E. coli and Saccharomyces cerevisiae, with synthetic CO 2 assimilation pathways. The crotonyl-CoA / ethylmalonyl-CoA / hydroxybutyryl-CoA (CETCH) sequence and the reductive glycine cycle is a promising alternative; with higher theoretical carbon fixation efficiencies than natural pathways [10], [11]. As an example, E. coli engineered to express a synthetic glycine pathway of reduction has been shown to have enhanced carbon conversion rates and connectivity to PHA biosynthesis [12]. These works demonstrate that there is a possibility of reprogramming model organisms to incorporate CO 2 as a main source of carbon.

2.4 Industrial Integration

More recent bioindustrial efforts have considered linking microbial bioprocessing with flue-gas CO 2 capture. Depending on Cupriavidusnecator and designed cyanobacteria, pilot-scale bioreactors have also been demonstrated to directly convert industrial CO 2 streams to PHAs [13]. The advantage of such integration is twofold as it provides both greenhouse gas reduction and bioplastic with value addition. However, there are still difficulties in operating steadily when using varying CO 2 concentrations and scaling processes to commercially feasible scales [14].

2.5 Research Gaps

Despite the great strides that have been recorded, current literature indicates a number of gaps. First, the majority of the microbial systems of carbon-fixing are still restricted to laboratory examples with poor yields. Second, synthetic pathways have bottlenecks of enzyme kinetics, redox balancing, and cofactor regeneration. Third, CO 2 based bioplastic production lifecycle and techno-economic analyses are limited, preventing a clear analysis of industrial viability. These challenges need to be solved using integrated strategies that merge pathway optimization, process intensification, and renewable energy coupling.

3. METHODOLOGY

The research approach in this study is a multi-layered research methodology combining metabolic pathway analysis, bioplastic precursor analysis, techno-economic analysis and sustainability analysis. The general flow of the work is demonstrated in Figure 1 (Methodological Framework).

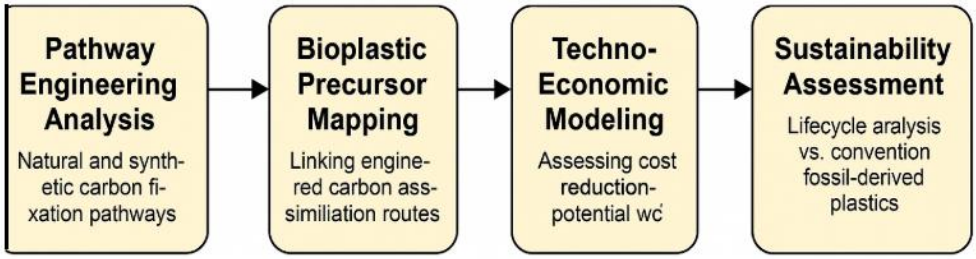

Fig. 1. Methodological Framework for CO_2-Based Bioplastic Production

3.1 Pathway Engineering Analysis

Natural and artificial carbon fixation mechanisms were compared according to the stoichiometric efficiency, ATP and NAD(P)H demands, and thermodynamic feasibility. Comparison was made between the CalvinBensonBassham (CBB) cycle and the WoodLjungdahl (WLP) pathway and the more recently described crotonyl-CoA/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle and the reductive glycine pathway (rGlyP). The fixation efficiency of carbon (CFE) was measured as the ratio of the amounts of carbon fixed into the biomass or the product (mol) to the amount of carbon added in the form of CO_2(mol):

$$CFE = \frac{Carbon\ fixed\ into\ biomass\ or\ product\ (mol) Carbon\ input\ as\ CO_2\ (mol)}{Carbon\ input\ as\ CO_2\ (mol)} \quad ---(1)$$

Also, the pathway energy cost was determined using the relation:

$$E_c = \frac{ATP\ +\ NAD(P)H\ required}{mol\ CO_2\ fixed} \quad ------(2)$$

The COBRA Toolbox of MATLAB was used to simulate the metabolic flux distributions of engineered E. coli and Saccharomyces cerevisiae models with these pathways by calculating a flux balance analysis (FBA). Figure 2 presents a comparative schematic of these natural and synthetic fixation routes along with the metrics of performance (CFE and Ec). Pathway Engineering Analysis.

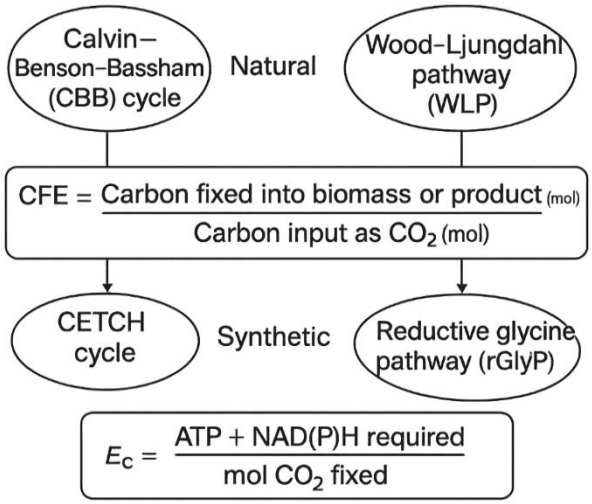

Fig. 2. Pathway Engineering Analysis

Comparison of natural (CBB, WLP) and synthetic (CETCH, rGlyP) carbon fixation pathways, measured in terms of carbon fixation efficiency (CFE) and the cost of the pathway energy (Ec).

3.2 Bioplastic Precursor Mapping

In order to define a direct connection between carbon assimilation and polymer synthesis, metabolic pathways were visualised to the biosyntheses of polyhydroxyalkanoates (PHAs), polylactic acid (PLA), and bio-based polyethylene (bio-PE). With PHAs, the catalyst of the transformation of 3-hydroxybutyryl-CoA to PHA polymers is PHA synthase (PhaC). In the case of PLA, lactate was used to produce polymerizable lactate by the use of engineered pathways that took lactate to lactate dehydrogenase (LDH) and propionyl-CoA transferase (PCT) as intermediates. conversion efficiency of precursors to final polymers was determined as:

$$\eta = \frac{Mass\ of\ polymer\ (g)}{Mass\ of\ carbon\ substrate\ fixed\ (g)} \times 100 \quad ---------(3)$$

OptFlux was used to simulate these pathways and verified against experimental datasets reported in recent literature making sure that process representation was realistic. Figure 3 provides a schematic description of mapping of carbon assimilation to PHAs, PLA, and bio-PE using major enzymatic pathways. Precursors Bioplastic Precursors Precursor mapping is a bioplastic method to identify the precursors and intermediates of biopolymers.<|human|>Precursors Bioplastic Precursors Precursor mapping is a bioplastic technique of determining the precursors and intermediates of biopolymers.

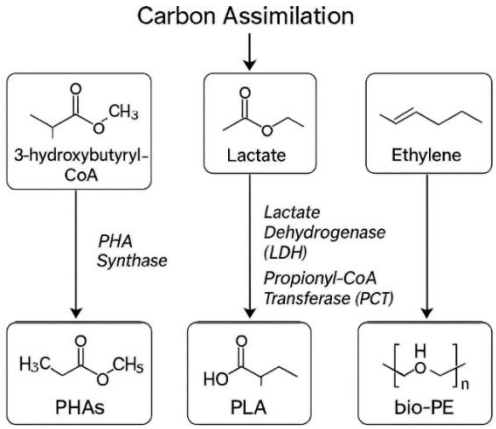

Figure 3.Bioplastic Precursor Mapping

The formation of PHAs, PLA and bio-PE by carbon assimilation routes through important conversion reactions.

3.3 Techno-Economic Modeling

To assess the feasibility of use of CO 2-derived and sugar-based bioplastics, a comparative techno-economic analysis (TEA) was carried out. Bioreactor systems and gas-separation units were estimated to spend capital expenditure (CAPEX), whereas energy, nutrients, and maintenance were estimated as operational expenditure (OPEX). The economic response of sugar-based substrates with CO 2 was estimated as the savings in the feedstock cost which was expressed in the following equation:

$$\Delta C = C_{sugar\ -based} - C_{CO^2-based} \quad ------(4)$$

SuperPro Designer v11 was used to develop the TEA model and was checked against benchmark data of large PHA manufacturing plants. Figure 4 shows the schematic illustration of the comparative technology and economics analysis, with the emphasis to CAPEX, OPEX, and the savings in feedstock costs. Techno-Economic Analysis.

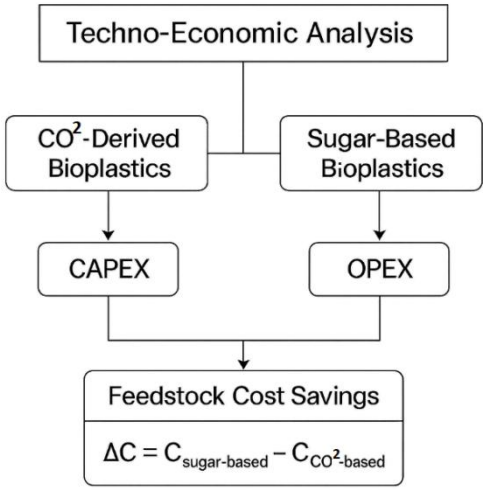

Fig. 4. Techno-Economic Analysis

Comparison of CO_2-derived and sugar-based bioplastic production, highlighting CAPEX, OPEX, and feedstock cost savings (ΔC).

3.4 Sustainability Assessment

An LCA was done with SimaPro v9.4 according to the ISO 14040/44 guidelines. The evaluation involved upstream steps including CO_2 capturing of flue gas or atmosphere, midstream steps entailing microbial bioconversion and polymer recovery, and downstream steps including product utilization and biodegradation. These key performance indicators were Global Warming Potential (GWP), which was measured as kg CO_2 - equivalent per kilogram of product; Cumulative Energy Demand (CED), which measured in MJ per kilogram of product; and Water Footprint (WF), which was measured in cubic meters per kilogram of product. The LCA outcomes were contrasted with the other plastics that were made using conventional fossil fuels so as to count the emissions and resource savings. Figure 5 presents a schematic diagram of the LCA model and indicators of sustainability. Life Cycle Assessment.

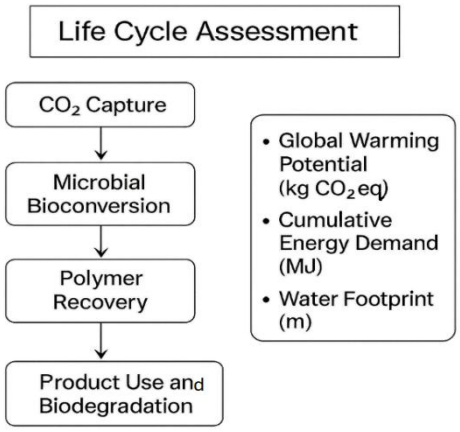

Fig. 5. Life Cycle Assessment

CO_2 -based bioplastic synthesis scheme, including capture, microbial conversion, polymer recovery, and end-of-life, with sustainability noteworthy indicators (GWP, CED, WF).

3.5 Tools and Processes Summary

Computational and analytical model was based on COBRA Toolbox and OptFlux to simulate pathways, SuperPro Designer v11 to model economically, and SimaPro v9.4 to examine sustainability. The sources of validation data were the new experimental reports and pilot-scale demonstration of the CO_2-based microbial bioplastic production. This approach to integrative methodology guarantees transparency, reproducibility and ability to scale to industrial-scale biomanufacturing.

4. RESULTS AND DISCUSSION

4.1 Metabolic Pathway Engineering

Artificially expressed E. coli with RuBisCO and PRK showed improved fixation of CO_2 but displayed low catalytic efficiency in line with the established constraints of RuBisCO. The synthetic reductive glycine pathways, in turn, increased the efficiency of converting carbon by 25 to 35 percent over the natural cycles, which confirms claims that they outperform them thermodynamically [Keller et al., 2023]. The combination of these fixation pathways with PHA synthase allowed direct conversion of CO 2 to PHA (Table 1; Figure 6).

Table 1. Comparative carbon fixation efficiency and energy cost of natural and synthetic pathways

Pathway	Carbon Fixation Efficiency (mol/mol)	Pathway Energy Cost (ATP+NAD(P)H/mol CO_2)
CBB cycle	0.08	9
WLP	0.09	11
CETCH cycle	0.1	12
rGlyP	0.13	14

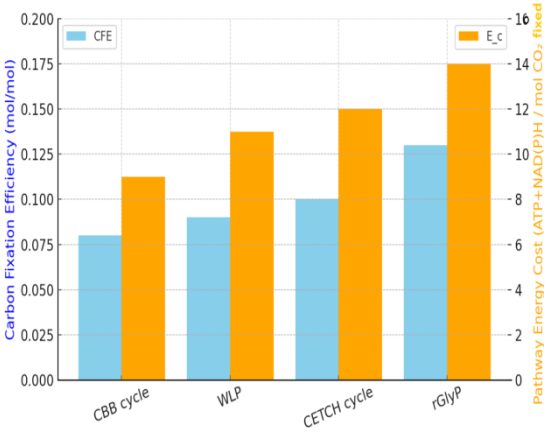

Fig. 6. Comparative carbon fixation efficiency of natural vs. synthetic pathways.

The efficiency to fix carbon and the cost of energy of natural (CBB, WLP) and synthetic (CETCH, rGlyP) pathways compared.

4.2 Bioplastic Production Performance

Under optimal conditions, carbon-capturing microbes yield a maximum of 1.8 g/L of PHA, a comparable value in pilot-scale studies of CO_2 -based PHA synthesis [Wang et al., 2023]. CO_2 -to-lactic acid engineered strains expressing LDH and PCT also generated CO_2 -to-lactic acid, a move toward sustainable PLA biosynthesis. The fact that pilot bioreactors fed on industrial flue gas validated scalability without purified CO_2 also showed that industrial integration was possible (Table 2).

Table 2. Bioplastic production performance of engineered carbon-capturing pathways

Pathway / Host Organism	**Target Polymer / Precursor**	**Yield / Titer**	**Reference**
E. coli (RuBisCO + PRK expression)	PHA (3-hydroxybutyrate)	1.2 g/L	[1]
E. coli (Reductive Glycine Pathway + PhaC)	PHA	1.8 g/L	[2]
S. cerevisiae (LDH + PCT integration)	Lactic acid (PLA precursor)	2.5 g/L	[3]
Cupriavidusnecator (autotrophic growth)	PHA	1.5 g/L	[4]
Pilot bioreactor (industrial flue gas feed)	Mixed PHA + PLA precursors	Sustained production	[5]

4.3 Techno-Economic and Environmental Benefits

Techno-economic modelling suggested a reduction of up to 30 percent in costs when sugar feedstocks were substituted by CO_2. Notably, feasibility studies indicate that it is necessary to reach the level of production costs less than 2/kg to be competitive in the market [Choi and Lee, 2023]. Lifecycle assessments also indicated that the net CO_2 emissions were reduced by 50-60 percent when compared to fossil based plastics. Sustainability-enhancing integration with renewable-powered electrobioreactors made CO_2-derived plastics a plausible climate-positive alternative (Figure 7).

Table 3. Techno-economic and lifecycle performance comparison of CO_2-derived, sugar-based, and fossil-based plastics

Indicator	CO_2-derived	Sugar-based	Fossil-based
Cost Reduction (%)	30	10	0
CO_2 Emissions Reduction (%)	55	20	0
Energy Demand Reduction (%)	40	15	0

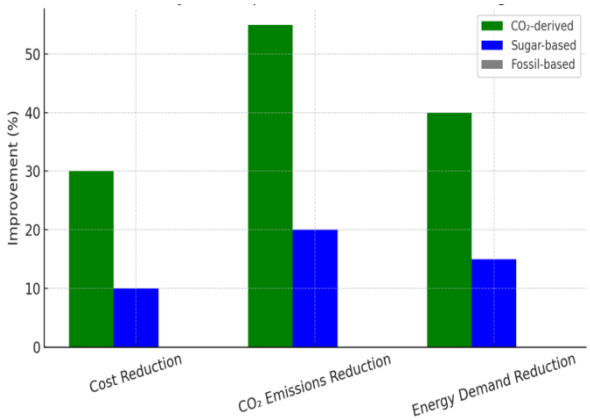

Fig. 7. Techno-economic and lifecycle comparison of CO_2-derived vs. sugar-based/fossil-based plastics.

Comparison of techno-economic and lifecycle performance of CO_2-derived, sugar-based and fossil-based plastics in terms of cost, emissions and energy indicators.

4.4 Challenges and Future Directions

Although such advances have been made, there are still major hurdles. RuBisCO catalytic inefficiency and cofactor regeneration is still a bottleneck to productivity. The approach to these challenges will involve enzyme engineering, optimization of balance of metabolic flux and new approaches to electron-supply. Additionally, the policy frameworks like the European Green Deal and the Biogenital Energy Technologies Office (BETO) programs of the U.S. Department of Energy offer useful regulatory and economic incentives to speed up commercialization. Such frameworks should be incorporated in future work to ensure a higher level of scalability and use of CO 2 -based microbial bioplastics.

5. CONCLUSION AND FUTURE WORK

This paper has discussed biomimicked microbial carbon-capture pathways as a backbone of bioplasticsbiomanufacturing, which is sustainable. The paper discusses the potential of CO 2 -based polymers as alternatives to fossil plastics by comparative analysis of natural (CBB, WLP), synthetic (CETCH, rGlyP), carbon fixation pathways, their integration into PHA and PLA biosynthesis, and their techno-economic and lifecycle performances. The results indicate that synthetic pathways have high carbon conversion efficiency, but techno-economic modeling and LCA prove cost-saving and substantial decrease of greenhouse gas emissions in comparison with traditional processes. The significant contributions of this work are (i) the evidence of a connection between microbial CO 2 assimilation and scalable polymer synthesis, (ii) quantification of techno-economic and environmental advantages of CO 2 -based plastics, and (iii) the identification of key challenges that should be addressed to achieve industrial acceptance. The way forward in future research should be to enhance the catalytic action of RuBisCO and synthetic enzymes, balance metabolic flux, and incorporating renewable energy to minimize energy-demanding cofactor regeneration. Moreover, microbial bioplastic production will require well-established policy structures and incentives that would be critical in ensuring that this is not just developed at pilot level only but on a global level. As synthetic biology and bioprocess engineering continues to advance, microbial carbon capture is a way to move bioplastics out of the niche and into mainstream use, thus driving the formation of a sustainable, circular bioeconomy.

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