

# Platform chemical production using cell-free biocatalysis

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Taylor E . Platform chemical production using cell-free biocatalysis. *J Environ. Microbiol* 2021;3(1):1-2.

## ABSTRACT

Host bacteria that have been genetically modified have a long history of producing certain proteins, as well as single enzymes for the alteration of chemicals made for industrial use by biological or chemical methods. These procedures have mostly been created through the process of discovery. The ability to design and assemble suitable enzymes into pathways that may or may not occur in nature to give a high-impact platform for the bio-manufacturing of chemicals, biofuels, and pharmaceuticals is a new development that is made possible by the synthetic biology method. Since whole cell synthesis is difficult or impossible to manage, a cell-free bio catalysis enables the manipulation of substrate ratios, the provision of regenerated cofactors, and the modification of high energy flux ratios. Here, we talk about the creation of cell-free bio catalytic pathways that may include additional free enzymes or multi-enzyme modules with heterologous catalysts. We review the state of commercialization-related applications while highlighting the significance of economical cofactor regeneration. However, issues still exist, especially with regards

to post-translational changes of proteins, such as glycosylation, phosphorylation, ubiquitination, acetylation, and proteolysis. Lists of the top value-added compounds from biomass, including glucaric acid, have been published by the National Renewable Energy Laboratory and Pacific North West Laboratory. There aren't many publications on how to create pathways for these useful intermediate chemicals using synthetic biology and cell-free protein synthesis. Despite at least one dedicated large-scale cell-free manufacturing of antibody conjugates, this observation remains. This review will outline one successful attempt at producing glucaric acid without the use of cells and assess advancements for additional important intermediate and platform chemicals.

**Key Words:** *Biofuels; Commercialization; Glycosylation; Phosphorylation; Acetylation*

## INTRODUCTION

The European Commission's circular economy strategy, strategic plan, and action plan were presented in 2014 and 2015, respectively. This has led to extensive discussion about the position, function, and relationship of the circular bioeconomy, as covered in detail by Carus and Dammer, as well as for a number of other distinct and primarily agricultural industries, including forestry, ethanol production, plastics, textiles, and animals, among many other examples. Absolute carbon efficiency is necessary for the circular bioeconomy, as is eventually moving away from the fossil feedstock's that the chemical industry relies on, primarily crude oil and other fossil resources like carbon and energy feedstock's. Sheldon and Woodley have provided a thorough analysis of the role that bio

catalysis plays in sustainable chemistry as a component of the circular bioeconomy and the new ability to create enzymatic transformations that adhere to specific design requirements. Using biotechnological methods, the energy sector may transition to mixed carbon sources, including conventional substrates (molasses and starch) and non-conventional feedstock's like CO<sub>2</sub>, methane, glycerol, and agricultural and urban waste streams, including CO<sub>2</sub> and plastic wastes. It is necessary to ask how cell free synthesis and synthetic biology may advance the goals of the global bioeconomy.

In synthetic biology and bio manufacturing, enzyme technology is crucial. The traditional idea of bio catalysis has evolved from single enzyme reactions to the inclusion of several enzymes permitting the creation of synthetic bio catalytic pathways as a result of developments in a number of instruments. A growing focus on bio

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Received: 4-February-2021, Manuscript No. PULJEM-22-5465; Editor assigned: 6-February-2021, Pre-QC No. PULJEM-22-5465 (PQ); Reviewed: 11-February-2021, QC No. PULJEM-22-5465 (Q); Revised: 16-February-2021; Manuscript No. PULJEM-22-5465 (R); Published: 19-February-2021, DOI: 10.37532/puljem.21.3 (1).1-2



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catalysis as an alternative to conventional pathways for sustainable and ecologically responsible bio manufacturing is the result of the present trend for "green" synthesis of chemicals. Although many enzymes are carefully regulated and have not naturally evolved to function under the most artificial industrial circumstances, enzymes are superb catalysts. In light of this, industry has relied extensively on chemical catalysts, albeit the use of enzymes is preferred over traditional chemical catalysts, especially for biopharmaceuticals where a high optical purity with a particular enantiomeric form is required. As a result, to supplement chemical catalysis for enantioselective phases in a pathway, individual enzyme reactions have been gradually introduced into industrial processes. Recently, several facets of this complex topic were examined, and Petroll et Alana lysis 's includes a more thorough explanation of the field's potential. The interested reader is directed to several accounts designed to address certain issues and circumstances that came up when transferring enzymes to industrial reactions.

Living cells (in vivo) are used in industrial bio manufacturing that incorporates single enzyme reactions because they offer the benefit of ongoing enzyme production and protection against enzyme degradation inside of physical barriers like cell walls or internal compartments. Because substrates and products may be diverted into various cellular metabolic pathways or exhibit toxicity to the host cell, multi-enzyme pathways in vivo present a more difficult situation and typically generate low yields. Continual cell-based bio catalysis is also hampered by the loss of recombinant plasmids that encode for pathway enzymes in hosts. In order to evaluate an enzymatic pathway without affecting the host organism's genetic makeup or being interfered with by other intracellular processes, multi-enzyme pathways are therefore put together with cell-free systems, allowing flexibility and control.