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'Hybrid' processing strategies for expanding and improving the synthesis of renewable bioproducts

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The versatile functionality of microbial biocatalysts offers a promising solution to the growing need to replace conventional, petroleum-derived fuels, chemicals, and materials with sustainable alternatives from renewable biomass. Whereas metabolic pathway engineering and strain optimization have greatly expanded the range of attainable bioproducts, it is by coupling microbial biosynthesis with traditional chemical conversions that the diversity of products that can ultimately be derived from biomass is truly beginning to reach its full potential. As will be the focus of this short review, such 'hybrid' strategies are now facilitating the generation of new and useful value-added bioproducts from renewable sources, the likes of which have previously been unattainable via biological routes alone.

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Introduction

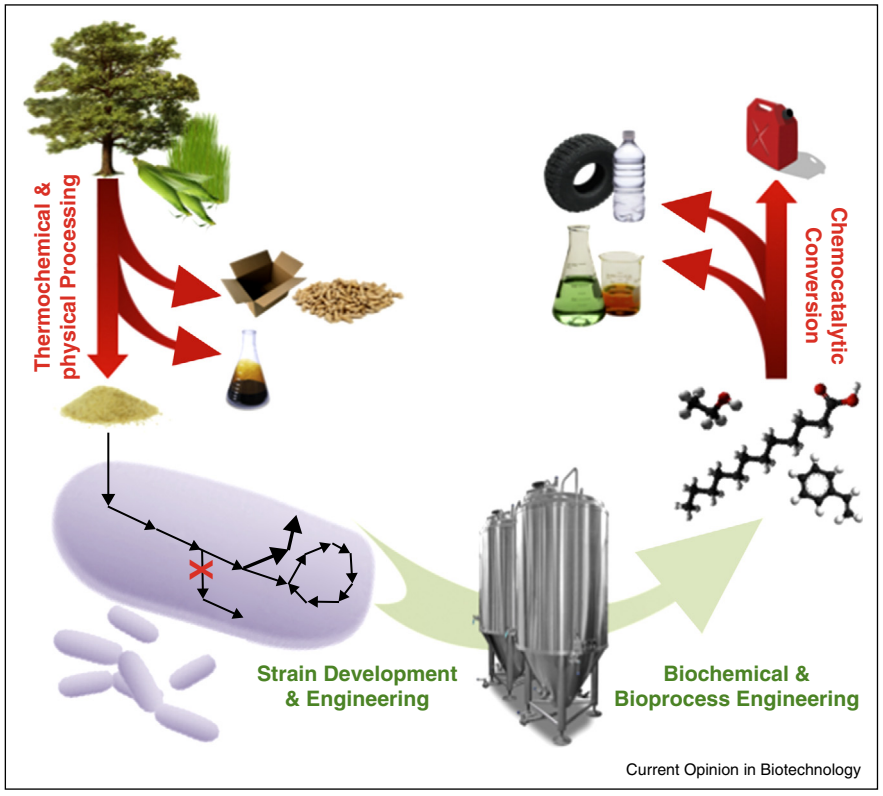
With few exceptions, non-renewable petroleum feedstocks currently represent the lifeblood of global fuels, chemicals, and plastics production. With worldwide consumption estimated at an all-time high (currently over 91.2 million barrels per day [1]), reliance on oil has never been greater or more widespread. However, concerns over rising environmental issues and the ever-increasing 'costs' associated with oil dependence–from both economic and geo-political perspectives–continue to drive interest in the development of alternative routes to conventional petroleum products from renewable and sustainable resources [2].

Biomass (i.e., harvestable plant crops) represents a promising and flexible feedstock for such purposes, and numerous strategies have been developed to recover and convert the reduced carbon and energy stored in biomass into desired end products. One promising strategy involves the use of microorganisms as biocatalysts to convert biomass-derived substrates–including carbohydrates, oils, and even proteins [3•]–into target compounds. In addition to enhancing the production of naturally occurring metabolites, advancements in synthetic biology and metabolic engineering have also further facilitated the rational engineering of non-natural enzyme pathways for the biosynthesis of novel bioproducts [4]. As a result, the breadth of products that can now be synthesized microbially has greatly and rapidly expanded in recent years [5].

Protein engineering/evolution efforts combined with continual advancements in next-generation sequencing techniques and the development of robust strategies for effectively mining genomes/metagenomes [6,7], as well as improved algorithms for in silico prediction of protein function [8•] have been and will undoubtedly remain as key contributors to the continued expansion of the repertoire of known and capable enzyme reactions. However, despite such progress, the number and diversity of products that can ultimately be synthesized from biomass feedstocks via enzymatic biotransformations alone will still continue to be limited by inherent restrictions on enzyme diversity. Thus, as an alternative approach, additional chemical transformations are instead increasingly being applied to convert biomolecule precursors synthesized by wild-type or engineered microorganisms into desired final product targets. In many cases, downstream chemocatalytic reactions can be implemented so as to effectively extend the function of an enzyme pathway, an approach that can be particularly advantageous if, for example: (i) the desired enzyme chemistry does not exist (or rather, is not yet known to exist), (ii) enzyme chemistries are known but lack performance due to either inherent (e.g. low turnover rates or equilibrium limitations) or context-dependent (e.g. poor heterologous expression) factors, or (iii) the reaction involves xenobiotic atoms and/or functional groups. In this way, chemistry can be employed to 'pick up where biology leaves off', allowing fundamental biological limitations to be overcome in a manner that complements biocatalyst phenotypes and expands product diversity.

One recent and commercially successful example marking this trend is the renewable production of the potent anti-malarial drug artemisinin. Whereas microbial biocatalysts have been engineered to convert sugars into

Figure 1



From biomass to renewable chemicals: 'hybrid' processing strategies integrate microbiological and chemical processes to convert and upgrade biomass feedstocks to useful and valuable products of interest.

precursors of the drug (i.e. artemisinic acid [9] or dihydroartemisinic acid [10]), it is a 'hybrid' process employing a final photochemical conversion step [11••] that is ultimately enabling Amyris Biotechnologies (together with Sanofi) to produce 50–60 tons of artemisinin per year, enough for 80–150 million treatments. The objective of this review is to illustrate how, by creating other novel and complementary interfaces between upstream biological and downstream chemical processes, the number and diversity of other important fuel, chemical, and plastic products that can be derived from renewable alternatives is continually and rapidly expanding (Figure 1). To this end, this short review will highlight a selection of recent examples in which hybrid processes have been successfully to the production of renewable biofuels and bioplastics.

Chemical upgrading of microbial lipids and fatty acids to drop-in fuels

Owing to their high energy density, lipids (i.e. triacylglycerides, or TAGs) and related aliphatic biomolecules represent ideal and versatile precursors for the production of biofuel alternatives to each of gasoline, diesel and jet fuels. Whereas native lipid biosynthesis is tightly regulated in most microorganisms [12], high-level production does occur in numerous oleaginous microorganisms, including bacteria, fungi, and yeast. Notable examples include *Acinetobacter calcoaceticus*, *Aspergillus oryzae*, and *Rhodotorula glutinis*, which can accumulate lipids at up to 38, 57, and 72% of total dry cell weight, respectively [13]. It should be noted, of course, that direct photosynthetic lipid biosynthesis has also been well studied in algae and cyanobacteria, as discussed elsewhere [13–15]. Meanwhile, elucidation and metabolic deregulation of lipid biosynthesis pathways coupled with further pathway engineering efforts has allowed achievable yields to approach maximal levels, while also resulting in the construction of new biosynthetic routes to other products with additional utility as biofuel precursors [16]. Such oleochemicals, the likes of which have been comprehensively reviewed by Lennen and Pfleger [17], notably include free fatty acids (FFAs) such as lauric, palmitic, and myristic acids. In contrast to lipids, FFAs–derived from native lipid biosynthesis via recombinant expression of appropriate (typically plant sourced) type I thioesterase [18] (a step that further specifies FFA chain length)–are naturally excreted through microbial cell walls, allowing for their facile recovery from the culture medium without biocatalyst disruption or the need for costly down stream processing

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