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Retooling microorganisms for the fermentative production of alcohols

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Bioengineering and synthetic biology approaches have revolutionised the field of biotechnology, enabling the introduction of non-native and de novo pathways for biofuels production. This 'retooling' of microorganisms is also applied to the utilisation of mixed carbon components derived from lignocellulosic biomass, a major technical barrier for the development of economically viable fermentations. This review will discuss recent advances in microorganism engineering for efficient production of alcohols from waste biomass. These advances span the introduction of new pathways to alcohols, host modifications for more cost-effective utilisation of lignocellulosic waste and modifications of existing pathways for generating new fuel additives.

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Introduction

The need for sustainable fuels from renewable biomass is driven by our dependence on depleting supplies of fossil fuels. This is compounded by concern over current and future energy security and reducing greenhouse gas emissions to alleviate climate change [1]. Biofuels offer an attractive and renewable alternative to petroleum-derived fuels, generated by fermentation of microorganisms on sustainable plant or waste biomass [2]. The market leader biofuel available is bioethanol, generated by yeast fermentation of sugar cane or cornstarch. It is typically blended with petrol (10–15%), although this is driven more by high levels of natural production in yeast, rather than its ability as an automotive fuel [3].

Classic first generation biofuels generated by fermentation of crop plants such as corn or sugar cane has a negative impact on food security, particularly when the fermentable biomass is derived from the edible parts of food plants. This led to the development of second-generation biofuels, where feed stocks are sourced from lignocellulosic non-food biomass, such as straw, sugar beet bagasse or other waste agricultural materials [2]. These wastes contain abundant levels of complex sugars in the form of cellulose, hemicellulose and other polymers, which are often inaccessible to microorganisms due to the absence of suitable saccharolytic enzymes [3]. Recent reviews have described the use of metabolic engineering to generate advanced biofuel components and improvement in waste biomass utilisation during fermentation [1,3,4•,5•,6].

Metabolic engineering of microorganisms has enabled the incorporation of non-native pathways to generate high-value drop-in alcohol biofuels. These fuels include linear and branched higher alcohols, such as butanol and propanol, which have higher energy content and lower hygroscopy than ethanol. In some cases, modifications of existing fermentative pathways can result in the production of different classes of biofuels. For example two *Escherichia coli* biopropane production pathways were recently described, utilising CoA intermediates derived from either a clostridial-like fermentative butanol pathway [7] or modifications of the fatty acid biosynthetic route [8].

This review will focus on recent molecular bioengineering of microorganisms for small scale *in vivo* ethanol and advanced alcohol biofuels production (typically <1 L). Initial discussions will focus on comparisons in the bioengineering of different microorganisms to generate specific classes of biofuels (linear and branched chain small alcohols/diols up to fatty alcohols), including novel chemolithotrophic and autotrophic pathways. This will be followed by a discussion on the recent approaches to increasing waste biomass utilisation (e.g. lignocellulosic waste) for increased biofuel production, grouped by organism type. It will cover both the engineering of novel pathways into new hosts and the modification existing pathways to redirect metabolism into new bio-alcohol production.

Engineered alcohol production pathways

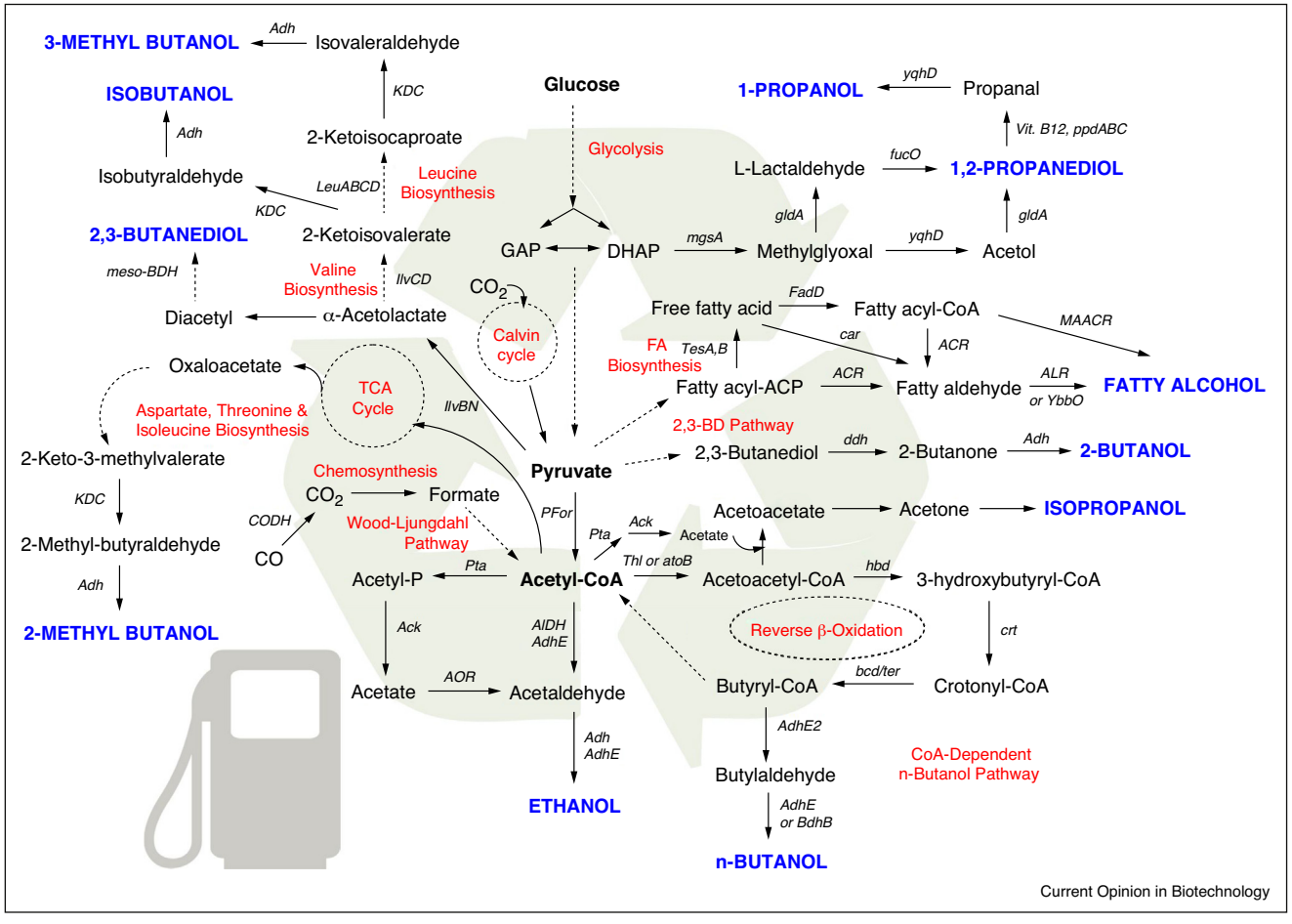
Bioethanol is the most dominant and commercially established biofuel. It is typically produced by plant biomass fermentation by *Saccharomyces cerevisiae* [9•] due to natural high levels of production and ethanol tolerance. Efforts

have concentrated on optimising ethanol production from lignocellulosic waste and other cheap, renewable biomass sources, and the production of alternative fuels by bioengineered microorgansims. Recent progress in engineering microorganisms for the production of alcohol biofuels (Figure 1), including the development of novel pathways and energy sources, is discussed here.

Autotrophic and photosynthetic alcohol production

Most biofuel production strategies are based on microbial fermentative pathways of plant and/or animal biomass. A recent study investigated the use of waste syngas (CO, CO_2 and H_2) for ethanol production by acetogenic bacterium *Clostridium autoethanogenum* [10]. Native ethanol and 2,3-butanediol pathways utilise CO for carbon fixation to acetyl-CoA via the Wood-Ljungdahl pathway. Ethanol production occurred via acetaldehyde using a bi-functional aldehyde/alcohol dehydrogenase (*AdhE*), or indirectly through acetate using aldehyde: ferredoxin oxidoreductase (*AOR*) and an alcohol dehydrogenase (Figure 1). Gene inactivation of *AdhE* generated a strain with an increased ethanol production (up to 180%) in lab scale trials. This study highlighted how simple manipulations of chemolithotrophs could generate potentially useful ethanol producers utilising waste industrial gases [10].

Figure 1



Overview of the recent engineered pathways for microbial alcohol biofuels production. Metabolic pathways are indicated in red, and dotted arrows indicate multiple steps are present. Enzymes are shown in italics, and the biofuels are in blue capitals. GAP = glyceraldehyde-3-phosphate; DHAP = dihydroxyacetone phosphate. Enzymes: *Ack* = acetate kinase; *ACR* = acyl-ACP reductase; *Adh* = alcohol dehydrogenase; *AdhE* = bifunctional aldehyde/alcohol dehydrogenase; *AdhE2* = bifunctional butyryaldehyde/butanol dehydrogenase; *AlDH* = aldehyde dehydrogenase; *ALR* = aldehyde reductase; *AOR* = aldehyde:ferredoxin oxidoreductase; *atoB* = thiolase; *bcd* = butyryl-CoA dehydrogenase; *car* = carboxylic acid reductase; *CODH* = carbon monoxide dehydrogenase; *crt* = crotonase; *ddh* = diol dehydratase; *FadD* = fatty acid CoA ligase; *fuco* = lactaldehyde reductase; *gldA* = glycerol dehydrogenase; *hbd* = 3-hydroxybutyryl-CoA dehydrogenase; *IlvBN* = acetohydroxyacid synthase; *IlvC* = acetohydroxyacid isomerase; *IlvD* = dihydroxyacid dehydratase; *KDC* = 2-ketoacid decarboxylase; *LeuA* = 2-isopropyl malate synthase; *LeuB* = 3-isopropyl malate dehydrogenase; *LeuCD* = 3-isopropyl malate dehydratase; *MgsA* = methylglyoxal synthase; *PFor* = puruvate:ferredoxin oxidoreductase; *ppdABC* = diol dehydratase; *Pta* = phosphate acetyl transferase; *TesA/TesB* = thioesterase; *Thl* = thiolase; *Ybbo* = aldehyde reductase; *YqhD* = aldehyde reductase.