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Production of fuels and chemicals from waste by microbiomes

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The demand for chemicals and fuels will continue to grow simultaneously with the costly requirement to treat solid waste, wastewater, and regarding climate change, carbon dioxide. A dual benefit is at hand if waste could be converted to valuable chemicals. The application of stable chemical producing microbiomes adapted to these waste streams may turn this challenge into an opportunity.

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Introduction

A microbiome consists of a mixed microbial community adapted to a natural or artificial environmental niche. Natural selection leads to efficient metabolic interactions among members of a microbiome, most often in association with the consumption of carbon and energy sources. It has been known for millennia that selection processes can be used to adapt microbial communities to synthesize useful products from waste materials. A classic example of such is anaerobic digestion (AD), where organic matter in wastewater is transformed into methane, thereby reducing a waste stream and gaining a valuable fuel [1,2].

Waste reduction is an increasing problem worldwide, particularly to remediate water and to avoid the release of carbon into the atmosphere. For example, the US EPA estimates that 3–4% of total energy use in the US is devoted to water treatment, and 45 million tons of greenhouse gases are emitted into the atmosphere every year from water treatment. In addition, there is a rapidly growing demand for more sustainable sources of chemicals and fuels. The following review describes recent advances that enable the production of valuable compounds in addition to methane from waste by microbiomes. While pure cultures of microorganisms in some instances perform these tasks, mixed microbial communities offer several potential benefits. For example, sterilization of a waste stream before microbial processing into useful compounds is cost prohibitive. Additionally, the physicochemical properties of the waste stream will potentially select the most efficient and effective microbial catalysts, and even lead to the evolution of more productive microbiomes. Finally, established microbiomes can be stable and resilient to adverse conditions and rapidly recover following an environmental upset.

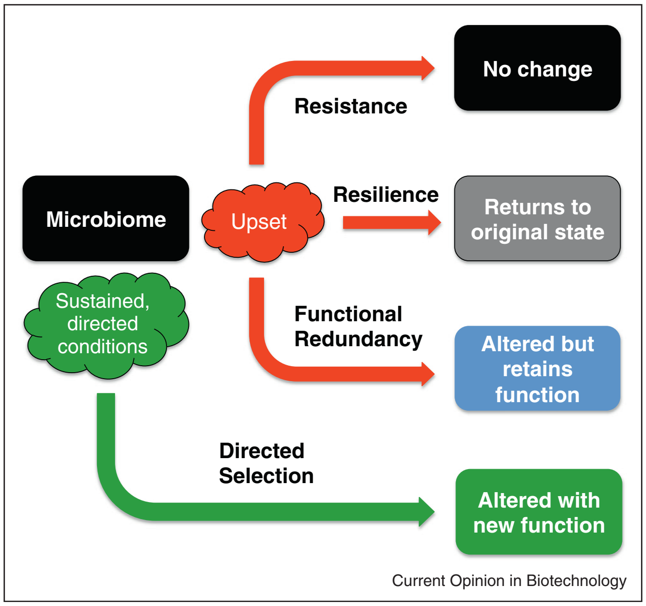
Resiliency, resistance, and redundancy of a microbial community, as defined by Allison and Martiny [3], are critical to maintain bioreactor productivity following an upset in conditions. Alternatively, an upset may be intentional and sustained, thereby altering the microbiome composition and performance toward the production of a preferred chemical (Figure 1). Indeed, such an approach has led to AD of waste to methane being a very successful bioenergy process [2]. Recently, Werner *et al.* [4••] completed an extensive community analysis of 9 full-scale AD systems treating brewery waste and determined by pyrosequencing of the 16S rRNA that each community possessed a unique, functionally redundant, and very stable microbiome. Furthermore, the researchers determined that the all-important syntrophic bacteria within these communities rebound following a disturbance, indicating that resilience is at work in AD systems. Other stable populations of microorganisms may be leveraged in the same fashion but under very different conditions to generate desired products. For example, Yates *et al.* [5] recently demonstrated that maintaining constant conditions in a microbial fuel cell selects for stable, reproducible generation of electricity through microbiomes dominated by *Geobacter* spp.

Chemical synthesis from waste streams by mixed microbial communities is now being extended beyond classical AD. Among the approaches now being explored are three exciting platforms that use microbiomes to produce useful chemicals from waste organic material or CO_2 (the carboxylate platform, bioplastic production, and microbial electrosynthesis, Figure 2 and Table 1). Each is in a nascent stage of technological development but may become the future of chemical and fuel production.

Carboxylate platform

The basis of the carboxylate platform is the production of carboxylic acids from waste organic material by a microbiome [6]. The products are typically short-chain carboxylates that are intrinsically valuable and may be further transformed biologically or chemically into fuels or more valuable compounds. The platform has been

Figure 1



Potential responses of microbiomes to an environmental upset or selective pressure.

comprehensively reviewed elsewhere [7••,8,9], but recent significant advances are described briefly here.

An important end product of the carboxylate platform is caproate, a precursor for biodiesel or fuel alkanes. Recently, a wastewater microbiome produced substantial amounts of caproate and caprylate from acetate with hydrogen and/or ethanol [10]. Chain elongation of ethanol to higher valued caproate was further improved with a microbiome adapted to un-distilled brewery wastewater [11••]. Product inhibition and methanogenesis were avoided by incubation at pH 5.5 and continuous extraction of the caproate. The result was a low energy extraction process in place of distillation, the avoidance of sterile fermentation conditions, and a yield of >2 g/L reactor volume day^{-1}, which is near the rates achievable with established bioenergy processes. In a separate report, Agler *et al.* [12] indicated that *n*-butyrate could be produced and harvested by a similar carboxylate microbiome; a potentially advantageous approach since the *n*-butyrate is more valuable than shorter chain carboxylic acids and could be converted into other industrial chemicals or fuels [10,13].

Alcohol-based fuels remain an important segment of the transportation infrastructure in North and South America and research has indicated that carboxylates may be converted into alcohol-based fuels. Steinbusch *et al.* [13,14] used microbiomes from upflow anaerobic sludge blanket (UASB) reactors treating distillery wastewater to convert carboxylates to alcohols. Since elevated partial pressures of hydrogen were needed to satisfy thermodynamic requirements, it is conceivable that wastewater first be treated using the carboxylate platform and remaining recalcitrant biomass (e.g., lignin) be gasified to syngas (CO, H_2, and CO_2). Syngas is a known substrate for autotrophic bacteria and can be fed to a community similar to that utilized by Steinbusch *et al.* to produce alcohols. These strategies offer many benefits over cellulosic ethanol/butanol production, including: first, remediation of municipal and/or industrial waste; second, does not compete for arable land; and third, complete conversion of organic waste (including lignin). The carboxylate platform-based MixAlco process operates similarly, generating a mixture of alcohols from complete substrate degradation [9]. A recent techno-economic analysis of production of alcohols or hydrocarbons by this process suggested a production cost of US $1.76/gal hydrocarbon (US $1.20/gal ethanol equivalent) at 400 tonnes per hour for a plant with internally produced H_2 [15•]. Several companies including Terrabon, IneosBio, Coskata, and LanzaTech are capitalizing on these benefits, commercializing syngas/carboxylate fermentation to ethanol.

The composition of carboxylate microbiomes remains somewhat mysterious, but modern high-throughput sequencing methods are now being used to examine metagenomes and metatranscriptomes of these complex communities. In the aforementioned study on caproate production [11••], metagenomic analysis determined that more than 50% of the assigned reads were from *Clostridium* spp. and that this genus dominated the chain elongation gene pool, 4% of which belonged to *Clostridium kluyveri* during maximum caproate production This observation is consistent with *C. kluyveri* dominating another caproate-producing mixed culture [10] and being capable of chain elongating carboxylic acids to caproate by a reversed β-oxidation pathway [16]. Agler *et al.* [12] demonstrated that changes in operating conditions, that is, pH control and continuous extraction of toxic product, led to an altered microbiome composition and enhanced production of butyrate in a thermophilic carboxylate bioreactor. Hollister *et al.* [17•] determined that carboxylate microbiomes consuming cellulosic wastes at 40°C were enriched in genes associated with hemicellulose degradation as well as the production of valerate and caproate, whereas a community maintained at 50°C was more specialized with genes for the uptake of cellobiose. Although different diverse communities were selected under the two temperatures, the metagenome data indicated that extensive metabolic flexibility and functional redundancy evolved and helped to stabilize each microbiome.

Bioplastics

Open microbiomes may also be used to synthesize bioplastics, more specifically polyhydroxyalkanoates (PHAs)