

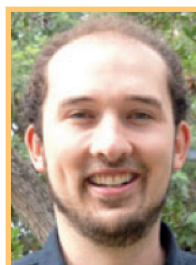
Synthetic biology approaches to biofuel production

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“...it is important for synthetic biologists to consider whether we can have a greater impact by focusing on drastically lowering costs rather than marginally improving yield.”

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The increasing scarcity, negative environmental effects and association of petroleum with unstable or politically hostile regimes have led to an urgent need to develop alternative fuels. In recognition of these issues, the US Congress has set lofty goals for the supplementation of gasoline with renewable fuels, mandating in the Energy Independence and Security Act of 2007 that by the year 2022 the US economy should be using 36 billion gallons of renewable transportation fuel per year, an enormous increase over the current production level of about 13 billion gallons [1]. Given the expanding market, it is not surprising that researchers, entrepreneurs and investors view this as a significant opportunity to develop novel engineering and biological approaches to the production of ‘green’ fuels. Biomass, due to its renewable nature and perceived abundance, is becoming an increasingly attractive fuel source.

However, this budding industry faces many formidable challenges. While a large portion of the renewable fuel mandate was originally to be met with cellulosic ethanol, this now appears unrealistic in the short term [1], and much time and money is being invested in the pursuit of various alternative resources [2]. Although

the total energy content of all the biomass on earth is almost unfathomably large, like sunlight it is diffuse and often difficult and expensive to concentrate. Furthermore, raw biomass is almost always found in a solid form with high water content, making it far more difficult and expensive to convey and process than a high-energy liquid such as oil. Most importantly, fuel is a low value compound, yet there is a tendency, particularly in the algae biofuel space, to prioritize high yields without sufficient regard to the ultimate cost [3–6]. When it comes to utilizing biological resources for the efficient production of renewable fuels, the focus should not be on whether it can be done, but more importantly on whether it can be done cheaply.

Although there has been a great deal of interest, an influx of funding and significant progress over the past decade or so, make it clear that innovative technologies will be required to produce large enough volumes of biofuels at a low enough cost to make this significant leap in the national consumption of renewable energy [7]. The successful approach to achieving these goals will most certainly be multifaceted, involving innovations in both engineering and biology, as well as modifications to

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existing infrastructure to support the storage and transportation of the next generation of fuels. Furthermore, success will require collaboration between scientists from many disciplines, with expertise ranging from molecular biology to mathematical analysis and traditional engineering. Here, we will focus on the use of synthetic biology to engineer organisms for the more efficient production of biofuel molecules, precursors or feedstocks, and we will discuss the potential benefits, the current hurdles and the importance of focusing on both yield and operational costs.

Synthetic biology, systems biology and postgenomics biology are terms that are increasingly encountered in the biofuels and biotechnology space. While they can mean different things to different people, here we use synthetic biology to describe the development of experimental and computational methods to describe gene networks from a more quantitative perspective, supplanting qualitative descriptions with more rigorous mathematical models [8,9]. Knowledge gained from this approach is used to develop gene circuits designed to perform specific functions, often by combining components from multiple organisms to generate novel functionality [10–13].

Mathematical modeling of gene networks is motivated by the enormous success this approach has had in other fields of science and engineering. The comparison between electrical and gene circuits is often made to point out how difficult it would be to describe an electronic device's behavior using only words. The implication is that describing biological networks mathematically will lead to knowledge of the system's behavior that would be impossible to predict from qualitative models. Therefore, the continuous refinement of a quantitative framework is likely to lead to the improved gene network design in biological systems, as it has for circuit design in electrical systems. Of course, it is important to note that biological systems are inherently more complex, variable and dynamic than their electrical counterparts and, therefore, present many unprecedented challenges.

On top of these complications, biological systems are able to evolve and mutate to dispose of disadvantageous engineered genetic circuits. This can be especially problematic in a biofuel context, as engineered metabolic pathways often divert carbon and energy to production pathways, resulting in reduced growth and poor health, and a cell can sense and eliminate these pathways in a matter of hours. However, if evolution is properly harnessed, it offers a tool that is unlike any available in other engineering disciplines. The ability to select for faster growing or higher yielding variants can greatly increase the speed of novel strain development and can achieve solutions that may not be possible or conceivable by rational design. While this could be considered 'dumb luck' to those more accustomed to

rigorous design, evolution is a powerful tool that should be harnessed rather than combated [14,15]. By combining this natural source of genetic variation with rational design and construction, there are endless possibilities for the creation of novel biological factories.

There are many potential contributions that synthetic biology can make to the pursuit of economical and renewable biofuels. The ever-increasing ease with which we can alter and genetically engineer new pathways in organisms provides the ability to 'reprogram' cells to produce molecules of interest [16]. This is often achieved by borrowing enzymatic machinery from one cell type for use in another, thereby combining critical pathways from different species to create novel metabolic networks [17]. As the ability to maximize an organism's energy efficiency is critical to achieving high yields, another popular approach is to 'rewire' cellular networks in a way that shifts the organism's priority from propagation to production, with the hope of creating an optimally efficient cellular factory [18]. Genetic modifications can also be employed to increase an organism's tolerance to conditions that are typically hazardous, such as extreme temperature or high concentrations of toxic biofuel molecules or precursors [19]. This is particularly useful if the organism is being engineered to secrete a molecule of interest, as the environment is inevitably going to become more harsh as the organism becomes more efficient.

However, synthetic biology has the potential to be more than just an extension to metabolic engineering, such as making a novel product in a microbial workhorse or increasing the yield of an important metabolite. While these are important and necessary goals, the field is far richer and is likely to have even broader impacts in the future. For example, an important focus of recent research has been on studying and modeling gene dynamics and cellular responses to environmental changes [20]. The study of genetics has traditionally been carried out in a static laboratory context, which fails to capture the richness of environmental diversity that organisms in the field experience every day. Genetic systems have evolved to cope and thrive in response to change, and a lack of understanding of genetic responses to environmental dynamics will hamper fields dependent on outdoor mass cultures, such as algae biofuels. Therefore, it is possible that instead of focusing on improving yields in a semi-sterile and expensive closed photobioreactor, synthetic biology could be more productive for improving the ability of a target strain survive in a highly dynamic and competitive but cheap open pond. That is, it is important for synthetic biologists to consider whether we can have a greater impact by focusing on drastically lowering costs rather than marginally improving yield.

It is clear that we are still in the early stages of engineering organisms for biofuel production, as manifested

by the many different organisms being explored concurrently for their potential to serve as factories [21]. *Escherichia coli* has been a surprisingly popular target, as unlike yeast it does not natively produce or tolerate high levels of ethanol. However, it is historically the most amenable organism to genetic modifications and it has already successfully been demonstrated to be able to use exogenous genes and hybrid pathways to produce foreign compounds [16]. Yeast, with a natural ability to produce and tolerate secreted ethanol, is an attractive possibility as well, and many researchers are pursuing the improvement of both the efficiency and the versatility of this approach. For example, there is much interest in the use of synthetic biology to introduce an ability to ferment nontraditional sugars or to increase the organism's tolerance to ethanol [22]. While algae species are extremely promising due to their ability to grow cheaply on little but CO₂ and sunlight, there is much less known about their genetic make-up, and their slower growth than traditional model microbes can be a hindrance to rapid progress. Nonetheless, there are many research groups and start-ups working on using algae to produce, and even secrete, high-value fuel precursors [18]. Even higher order plants are being investigated and modified to improve their potential as feedstocks for enzymatic conversion to ethanol and other biofuels. As the primary hurdle in this biofuel avenue is the difficulty in fully breaking down the lignocellulose in the plant cell walls, synthetic biology applications are being explored to reduce the resistance of cell walls to enzymatic deconstruction [23].

As we begin to see the interest in synthetic biology and its potential for biofuels applications increasing, there are many challenges and potential hurdles, both logistical and ideological, that the use of genetically modified organisms presents. The most important factor that will determine the success of the biofuels industry is the economics: whether we can produce enough biofuel for a low enough cost to make the use of renewable fuels a reasonable choice for the consumer. In the case of microbes that will be used to produce fuel or precursor molecules, the cost and source of the organism's feedstock is critical. The carbon sources required to fuel these factories must be taken into account at an early stage to avoid potentially insurmountable costs or controversy, as the corn ethanol 'food versus fuel'

debate has demonstrated. The competition for limited water and land is only going to grow stiffer, so a balance or even a collaboration must be struck in which perhaps biofuel producers use discarded food byproducts as feedstocks. In the case of photosynthetic organisms that do not require carbon-based feedstocks, land and strain fitness are the issues that will likely determine cost. Genetically modified strains will inevitably suffer from a loss of competitiveness with native, wild-type strains, making open outdoor growth a significant challenge. Generally, this known issue is being addressed either through the use of tightly controlled and sterilized environments or selective environmental conditions, either of which can be prohibitively expensive at a large scale.

Beyond these technical hurdles, safety concerns over using genetically modified organisms on a large scale, even amongst the scientific community, are still fairly widespread. The stigma on genetically modified organisms will continue to loom over the biofuel industry until we develop guidelines and infrastructure for the approval of their use when properly safeguarded. One problem is that there is still no defined means to regulate and approve their use, and there are no set standards for what is acceptable. If the government is going to mandate the production and consumption of biofuels, they will need to provide support and resources to the scientists working to meet the goals, as well as to involve scientists in the development of new policy. Overall, the potential for developing and using engineered organisms for the production of sustainable fuels is extremely promising. While the challenges that we face are daunting and the road ahead will be long and arduous, with patience and a collaborative spirit the potential payoff will be huge for researchers, entrepreneurs and society as a whole.

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