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Review

In vitro synthetic enzymatic biosystems at the interface of the food-energy-water nexus: A conceptual framework and recent advances

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ABSTRACT

The food-energy-water (FEW) nexus is interconnected and interdependent and provides a physical foundation for mankind. The production of safe food, renewable energy, and clean water through biological means, especially microbial bioconversion, has attracted an enormous attention worldwide. Recently, in vitro synthetic enzymatic biosystems (ivSEBs) comprised of numerous enzymes and coenzymes, as a disruptive biomanufacturing platform, has been proposed and demonstrated to address key challenges at the interface of the FEW nexus. Light, electricity, and hydrogen can provide energy to fix CO_2 and produce food and biomass. Lignocellulose-derived cellulose can be converted to starch and biofuels. Starch can be further converted to bioenergy, including electricity, hydrogen and liquid fuels. These high-energy efficient bioprocesses lead to significantly less water usage and also can be used to reduce water pollution. In this review, the conceptual framework and latest advances of ivSEBs in the FEW nexus are summarized. Their limitations and future research directions on the design and improvement of ivSEBs are also discussed.

1. Introduction

Basic demands for food, energy, and water are rapidly increasing, driven by a growing economy and a rising population. Meeting these growing demands, however, presents tremendous challenges due to the competition for limited resources (e.g., land, water, energy, and natural resources) amid aggravated environmental issues [1,2]. We need to rethink our current manufacturing processes at the interface of the food-energy-water (FEW) nexus [3,4]. Systematic analysis and paradigm-shifting solutions are necessary for sustainability.

Biomanufacturing that produces commercially important biomolecules by utilizing biological systems as biocatalysts has played a significant role in the agricultural, food, material, energy, and pharmaceutical industries [5]. Such biological systems refer to whole cells (e.g., microorganisms, animal cells, and plant cells), cell extracts, enzymes, proteins, and tissues. Benefiting from renewability and modest reaction conditions of the biological systems used, biomanufacturing allows for a decrease in carbon emissions, energy consumption, water use, and solid waste while minimizing impacts on water security and biodiversity [4,6]. With the advances in molecular biology in the past several decades, metabolic engineering- and synthetic biology-driven microbial bioconversion (through in vivo biosystems) becomes dominant in biomanufacturing nowadays.

In vitro synthetic enzymatic biosystems (ivSEBs) have emerged as a third state-of-art biomanufacturing platform, independent of whole cell fermentation and classic enzyme biocatalysis [2]. ivSEBs are comprised of numerous enzyme components and/or coenzymes for implementing complicated bioreactions (Fig. 1A) [7,8]. Differing from the use of cell extracts or multi-enzyme cascades that are designed for the production of high-value proteins, peptides, metabolites, and other fine chemicals, IvSEBs focus on the production of relatively-low-value biocommodities, including bioenergy, bulk biochemicals, and food [9–11].

In comparison with the predominant in vivo biosystems, ivSEB offers multiple advantages: (1) high product yield and energy efficiency due to a lack of byproducts and cell mass; (2) high volumetric productivity due to the lack of a cell membrane and/or high volumetric catalyst loading; (3) broad reaction ranges and high tolerance to harsh conditions; (4) great engineering flexibility, i.e., the ability to fine-tune the number of exchangeable biocatalysts, easy assembly of the various biocatalytic modules, and the ability to avoid competing metabolic pathways; and (5) the capability of implementing unnatural reactions that living cells cannot perform. However, it is worth noting that ivSEBs cannot completely replace whole-cell fermentation; for example, ivSEBs cannot produce bulk enzymes, nor are they suitable for the production of complicated secondary metabolites [9,12].

Although still in the early stages, ivSEBs have been demonstrated to

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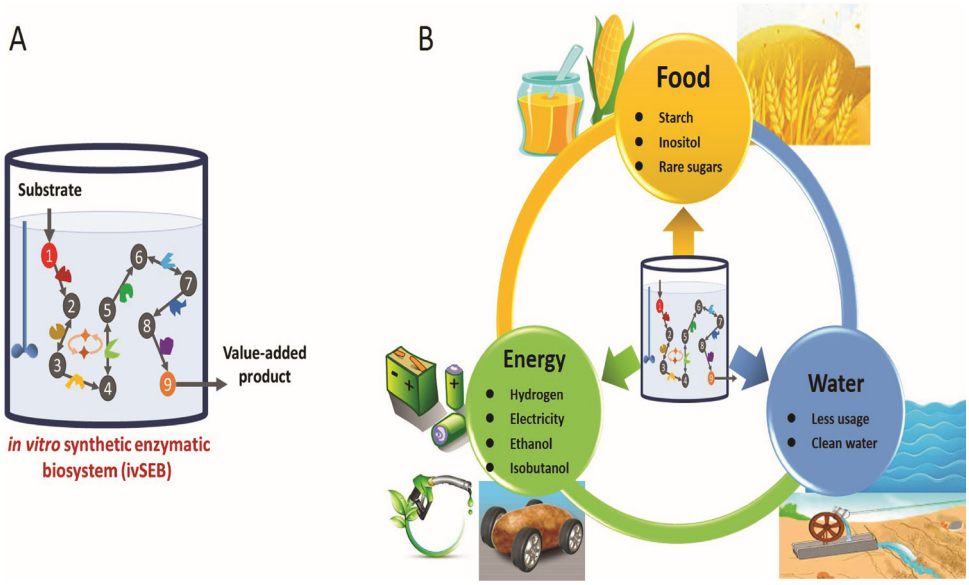

Fig. 1. Schematic of an in vitro synthetic enzymatic biosystem (ivSEB) (A), where the numbered round circles represent molecules and the irregular shapes represent enzymes, and its application at the interface of the food-energy-water nexus (B).

produce an increasingly number of products, such as hydrogen, electricity, liquid biofuels, food and feed ingredients, and precursors for materials, drugs, or other biochemical, since the last decade [13–18]. These examples could have a significant impact on the FEW nexus and open the door to a cleaner, healthier, and more energy-efficient world in the future (Fig. 1B). In the next four sections, we will discuss a conceptual framework and the application of ivSEBs at the interface of the FEW nexus in detail, followed by the analysis of their current drawbacks and possible solutions.

2. Production of food and biomass

Food security and availability is a top government priority. Uneven food distribution, natural disasters, technology levels, and the geographic environment pose great challenges, especially in developing countries [19]. Modern food production practices are water and energy intensive. Agriculture, including irrigation, pesticide and fertilizer application, crop cooling, and frost control, account for approximately 70% of global fresh water usage [4,20]. For example, the production of one kilogram of wheat requires the use of ~1300 kg of water, while 1 kg of beef requires 10,000–20,000 kg of water [21]. At the same time, much energy is required to pump water, power machines, and transport goods. Over the past decades, innovative agricultural technologies have been developed to decrease input and maximize output, such as genetically modified crops, fertilizer deep placement, high-roofed greenhouses, precise agriculture, and farm management information systems [22].

In recent years, several artificial food products have been made in the laboratory rather than on farms; for example, cultured meat produced in vitro by tissue engineering and artificial milk protein produced from yeast cells [23]. Besides, an in vitro pathway to produce artificial starch (a polysaccharide) from renewable cellulosic material (another polysaccharide) has been validated via a three-step ivSEB (pathway 3→1, Fig. 2A) [24]. In detail, cellulose is partially hydrolyzed by cellulase into cellobiose, followed by a conversion of cellobiose to glucose 1-phosphate via cellobiose phosphorylase. Glucan phosphorylase continuously adds one glucose unit from glucose 1-phosphate to the non-reducing ends of amylose, yielding a long-chain starch. Presently, the conversion yield from cellulose to synthetic starch is still too low, plus the high processing cost and limited product titer, suggesting that there is a long way to make this technology economically feasible. If successful, given the annual cellulose production of 100 billion tons, enzymatic transformation of a very small fraction of cellulose to starch would drastically enhance food security, as approximately 2.5 billion tons of starch-rich cereals are currently produced. In addition to harvesting starch-rich seeds from annual grains, another option would be to harvest cellulose-rich perennial plants. This paradigm-shift would have a higher biomass yield per hectare, reduced chemical runoff, better water conservation, and less energy input [25]. In addition, it becomes possible to grow such cellulosic plants on low-quality or marginal land, leading to a dramatic increase in “arable” land availability. Besides, this artificial starch can be further manipulated simply by adjusting enzyme loading, ratio, and source, in order to generate various chain lengths or very low branch frequency. Such linear amyloses produced would be used as resistant starch for high-end applications such as dietetic food as well as drug delivery and coating [26].

Besides using renewable cellulosic substrate, though ivSEBs, more food and biomass are envisioned to be produced from CO_2 in bioreactors energized by artificial photosynthesis [27]. Sunlight can be converted to electricity by photovoltaics, which then can be used to obtain hydrogen through the electrolysis of water. Electricity or hydrogen can both be used to generate reducing power, which can enter the enzymatic module for carbon rearrangement (Fig. 2B) and for sugar activation (Fig. 2A). For example, a hypothetical pathway 9→1 has been designed to efficiently use electricity to fix CO_2 and generate starch. In another report, a novel artificial photosynthesis pathway has been devised using 21 enzymes [28]. In general, the CO2 fixation mediated by formate dehydrogenase and formaldehyde dehydrogenase can generate formaldehyde, which is further converted to fructose 6-phosphate by 3-hexulose 6-phosphate synthase and hexulose phosphate isomerase from the ribulose monophosphate pathway [29]. The ribulose 5-phosphate regeneration is catalyzed by enzymes in Fig. 2B and amylose can be produced from fructose 6-phosphate via enzymes in Fig. 2A. Compared to the low efficiency of natural photosynthesis (0.3% for plants and 1% for algae), this ivSEB could have an overall energy efficiency of approximately 15%, with a solar-to-electricity efficiency of 18% and an electricity-to-starch efficiency of nearly 80% [30]. This hybrid system could surpass natural photosynthesis by saving land and water use by over 1000-fold as well as addressing the problem of product separation from the aqueous phase [28]. Recently, a synthetic crotonyl–coenzyme A (CoA)/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle for the continuous fixation of CO_2 in vitro via a reaction