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Synthetic Biology: Approaches, Opportunities, Applications and Challenges

Aasia Bibi[1] *, Amer Ahmed[2]

[1]*Department of Biochemical Sciences, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy*
[2]*Department of Life Science, University of Siena, Via Aldo Moro 2, 53100, Siena, Italy*

Abstract

Synthetic biology (SynBio) is a very vast field of research that produces new biological parts, appliances, and systems. It is the application of engineering principles to design and construct new bio-based biologicals, devices and systems that exhibit functions not present in nature or to redesign the existing systems to perform specific tasks. Synthetic biology varies from other disciplines including system biology, biotechnology and genetic engineering. For instance, while system biology focuses on obtaining a quantitative understanding of the naturally existing biology systems, the synthetic biology focuses on engineering, designing, and synthesis of new novel biological functions utilizing the biological information drawn from systems biology analysis. SB utilizes computer algorithms to alter genetic sequence before synthesizing them in the laboratory. Moreover, SB employed gene shuffling and refactoring tools that may alter thousands of genetic elements of an organism at once. In the present article, we aim to discuss the basic approaches of synthetic biology. Furthermore, the application of synthetic biology on biomedical science, drug discovery development, bioenergy and agriculture will also be discussed. Finally the challenges facing the researchers in the field of synthetic biology such as those technical, ethical and safety will be also highlighted.

Keywords: SB; Synthetic Biology, Bio-based Biological, DNA recombinant technology, Biomedical applications

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Corresponding author:
aasia.bibi@uniroma1.it

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Contents

1. INTRODUCTION

Synthetic biology, abbreviated as (SB) or SynBio, the application of engineering principles to design and construct new bio-based biologicals, devices and systems that exhibit functions do not present in nature or to redesign the existing system to perform specific tasks [1]. For example, having the characterized part (amino acids, bases, proteins, genes, circuit and cells), right software, brains and sufficient quantitative biological and molecular knowledge about the target system, it is possible to design bacteria that produce ethanol from water, CO_2 and light [2, 3]. On the other hand, SynBio in engineering disciplines that utilizes standard parts such as (gene, protein) and put them together using bioinformatics and simulation tools to construct genetic/biological circuit to perform or modify a biological function. SB relies on DNA recombinant technology, DNA sequencing and synthesis, high through-put technologies (NMR, OMICS, and Microarrays) and modelling and simulation. SB synthetically constructs artificial cells, ribosomes, DNA strands, nucleic acids, and genetic circuits which are not found in nature and cannot be obtained from existing organisms. SB utilizes computer algorithms to alter genetic sequence before synthesizing them in laboratory. Moreover SB employed gene shuffling and refactoring tools that may alter thousands of genetic elements of an organism at once. Now a days, synthetic biologists are using whole genome assembly tools to construct an entire microbial genome replacing the traditional gene gun or vector techniques utilized in traditional recombinant DNA Technology [4-6].

It is thought that the first scientist to coin the term synthetic biology was the French chemist Stéphane Leduc (1853-1939). However, some believed that Waclaw Szybalski was the first to use the term synthetic biology in 1974 suggesting that recombinant DNA technology will allow the construction of cell with modified genetic material to perform a specific function or to test the hypothesis of biological functions; this field becomes latterly known as Genetic engineering. The current synthetic biology research with a new definition is a new discipline of around 1.5 decades [7]. In 2003, Craig Venter Institute (USA) created the first synthetic virus and in 2008 first complete chromosome composed of 1000000 bp was designed. By May 2010 the same institute announced for the construction of first self-replicating synthetic bacterium, named as Mycoplasma mycoides JVC-syn1.0; Synthia [8, 9].

Today, synthetic biology is global and well financed with products already present in market places. For example, 2% of US GDP is based on synthetic biology products and the global market of synthetic biology products reached US$2.4 billion by 2013, with applications ranging from medicine to agriculture.

2. SYNTHETIC BIOLOGY: APPROACHES

Synthetic biology attempts to engineer the biological systems for various applications of human needs. It combined the genetic design with genetic engineering to construct or modify biological systems. Generally synthetic biology has two main approaches top-down and bottom-up approaches (Fig. 1).

Top-down approach: This approach focuses on reengineering the pre-existing natural living systems for some desired purposes. It may include synthesizing or transplanting entire genomes. This approach addresses the questions regard desirable and undesirable potential interactions between the constructed subsystem or genetic circuit and its biological context. It also focuses on development of strategies for harnessing or compensating for these influences. This approach is considered less challenging and takes advantages of massive accumulated biological information of the concerned organism into successful engineering synthetic organism wit new desirable trait of human use [10, 11].

Bottom-up approach: This approach, also known as “protocells”, attempts to make new simple kind of minimal cellular life using raw ingredients that were never alive. It creates new self-replicating living cells such as *Mycoplasma mycoides* JVC-syn1.0; Synthia through designing and assembling simple component into construction of entire cells with new and predicted properties not found in nature. Bottom-up approach characterizes single individual modules (may be parts or subnetworks of parts) and their assembly into novel configurations in isolation from the endogenous cellular context into which they will eventually be placed. Subsequently, this approach addresses questions regards desired function of intended synthetic system and parts that used to construct this system (e.g., promoters, ribosome binding sites, etc.). The configuration of the designed modules and its predicted behavior are also important question of bottom-up approaches. Bottom-up is technically hard and the technology is extremely tedious, although progressively improving [12-14].

It should be noted that both top-down and bottom-up synthetic biology are blending and becoming harder to separate even it is crucial to distinguish them for ethical, social and regulatory challenges which have been raised against top-down approach and expected to be raise against bottom-up approaches [12].

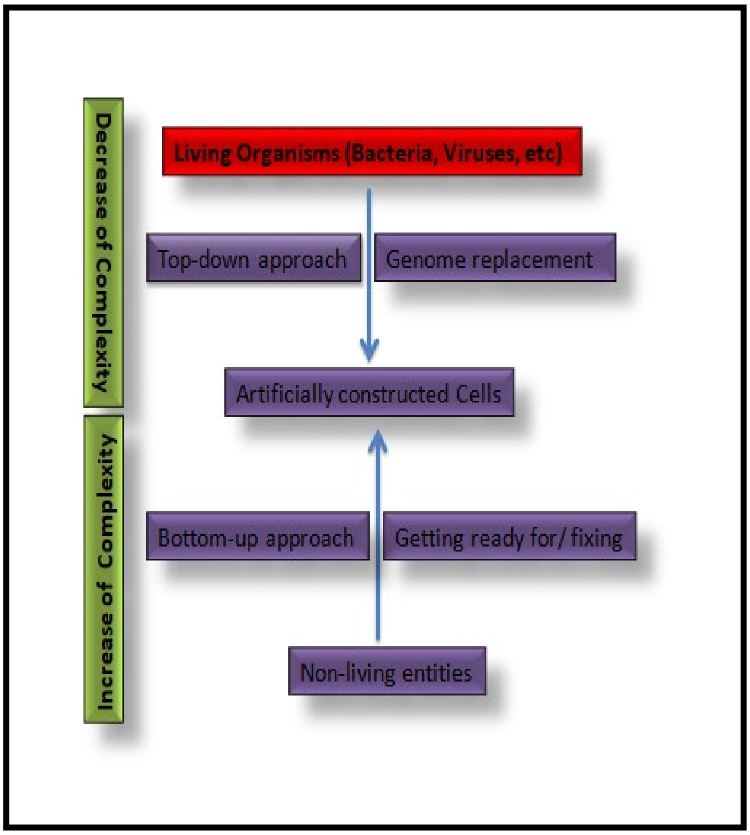

Fig.1. Top-down and bottom-up approaches for Synthetic biology [15].

3. SYNTHETIC BIOLOGY: OPPORTUNITIES

Synthetic Biology is the discipline of science at the interface of biology, chemistry, information science and engineering which has tremendous applications in biomedical science, bioenergy and environment, drug discovery and development, and agricultures.

4. SYNTHETIC BIOLOGY AND BIOMEDICAL APPLICATIONS

Early pioneering studies of synthetic biology have been focusing on engineering and standardization of prokaryotic cells. More recently SB investigation on mammalian and yeast cells have been significantly emerged for medical purposes. Perhaps the biomedical applications of synthetic are considered the most potential and promising. These studies are applying synthetic biology principles to mammalians cells to understand diseases, mechanism of action and genetic circuit-oriented therapy of complex diseases 16. ON-OFF Switches such as transcriptional, post-transcriptional, and translational and post translational switches have been described. The synthetic gene network for therapeutic purposes such as cancer treatment, immuno-regulatory networks, among others, have also been described [17]. Moreover, through Synthetic biology, scientists are synthesizing, assembling, shuffling and mutating individual bacterial and viral genomes to understand the molecular mechanism of host-pathogen interactions and diseases mechanisms. The genome of H1N1 virus has been reconstructed and functional studies have revealed new insight into the key virulence factors of the pathogen: namely, a haemagglutinin variant that induces membrane fusion without trypsin activation and a modified polymerase that enhances viral replication [18]. Other viruses have been studied using synthetic approaches includes SARS-like bat coronavirus, HBC and HIV [19]. Synthetic biology principle has also been applied to understand complex disorders of immune system such as dysfunction of B-lymphocyte activation [19]. Another avenue of Synthetic biology in biomedical science is the design and construction of attenuated pathogens and their parts e.g., protein that can be used as vaccines [20]. For example, DNA synthesis and assembly have produced a safe live vaccine against the poliovirus. This vaccine was produced by genome scale changes in adjacent pairs of codons from over to under-represented codon in viral capsid genes which resulted in reduced expression and impaired viral replication with enhanced immunization in mice [21]. Similarly, synthetic biology approaches are applied to design viral strain harboring conditional dominant-lethal synthetic circuit which used to control the transmission of parasitic diseases such as malaria and dengue fever viruses [19, 22].

Microbiota is a community of microbes (bacteria and fungi) that live in association with multicellular host in symbiotic or commensal relationships and which causes diseases when their balance is disturbed. Microbial communities vary significantly from one niches to another e.g., oral cavity and digestive tract [23]. Instability of human gut microbiota have been associated with diseases such as irritable bowel syndrome (IBS), obesity, cancer, and neurological disorders and that of skin is associated with acne and dandruff [24]. The utilization of probiotics or prebiotics is the earliest form of microbial therapeutics with the goal of assisting or maintaining a healthy human microbe's composition. Synthetic biology; a rapidly growing field that aims to design and achieve programmed cellular behavior using natural and synthetic biological component, has been accelerated through employment of NGT, construction of genetics circuit, synthetic cell-to-cell communication and CRISPR-CAS genome editing tools. For the field of microbiomes, SB renders tools to investigate structure–function relationships among microbiota and engineer novel biotic therapeutics and the development of microbes which sense and respond to their local environment. Numerous studies have been carried out to engineer the commensal and probiotic lactic acid bacteria, bifidobacteria, and *Bacteroides* for probiotics application and are well reviewed recently in [25, 26].

In the recent past years, synthetic biology was considered to be a discipline of engineering for biological systems. To carry out natural biological systems, substrates are required, if we do not manipulate substrates, it will be incomplete understanding of biology of the whole organism. Synthetic biology has played a great role in this regard to understand the challenges that come across for rational and high-throughput designing. The complete set of design for biological system includes construction of DNA, computational designing tools, libraries part, manipulating and probing synthetic circuits [27]. Synthetic biology functions to produce different devices, organisms and systems with beneficial functions on the basis of biological building blocks. Initially they were produced to confirm the reliability of simple processes. Newly designed devices now can contribute to understand the mechanism of disease, enable good production of therapeutics and allow to design novel procedure for the treatment of cancer, diabetes, immune diseases and metabolic disorders [28]. Hydrogels are the materials having different shapes attributed by the presence of high amount of water with different physical properties. They can be produced in order to resemble the extracellular environment of body tissues that enable their use in biosensors, drug discovery and development. Hydrogels are very important in the area of stem cell research, cancer biology, cell morphogenesis. Furthermore, hydrogels are being used in clinical applications in cell therapy, engineering of tissue and biomedical research [29]. Synthetic biology is the science put together again standard biological things in a well defined and rational way to create and engineer functional biological designer devices, systems and organisms with beneficial and useful, preferably therapeutic functions. Synthetic biology has significantly advanced the design of complex genetic networks that can reprogram metabolic activities in mammalian cells and enhance the synthesis of novel therapeutic strategies for future gene-based and cell-based therapies [30].

Nanotechnology and molecular biology has played a great role in the field of emerging research areas such as: magnetic nanoparticles, nanobiotechnology. In these fields nanoparticles are employed having specific size, shape and ability to be incorporated externally, and they increase the process of Magnetic Resonance Imaging (MRI). These nanoparticles have many uses in the field of biology and medicine such as: protein purification, drug discovery and development and medical imaging. Due to a lot of benefits and enhanced use of nanoparticles in biomedical applications, there is a need to construct magnetic nanoparticles having many functions. There are two methods to fabricate magnetic nanoparticle-based multifunctional nanostructures. First function at molecular level describes about adherence of antibodies, proteins and dyes to the magnetic nanoparticles [31].

Other method involves quantum dots (QDs) or nanoparticles made up of metals. These nanoparticles have many applications and more than one function in the field of biomedical, such as: binding with specific ligands, antibodies, or proteins, magnetic nanoparticles are highly selective in their binding modes, such nanoparticles can be applied for the purification of proteins, detection of bacteria and decorporation of toxins, this can lead to applications in nanomedicine [32]. Polymeric nanofibrils play a great role in the field of biotechnology and biomedicine, specifically the nanofibrils having natural and synthetic polymer sources, have an important role in tissue engineering and regenerative medicine [33]. Ultrasound-power nanowire motors, having ability to work with bioreceptors and a drug containing polymeric partition are used to capture, transport and delivery of drugs [34]. Now a days, biomedical engineering is considered to be fastly increasing field in research areas, specifically in the field of drug delivery, transfer of gene, engineering of tissues and regenerative medicine. One can design biomaterials having multiple functions and biologically active. It has many benefits: introducing chemical functionality, interaction between proteins and genes, modified bioactive molecules are obtained having better biological properties such as interaction of cell materials and their degradation, increasing mechanical properties and provision of building blocks involved in metabolism [35]. Metal ions and metal complexes are most commonly used in nature. Metal ions comprises of 1% of human body by weight, while the trace ions comprises of approximately 0.01% by weight. Many transition metals show a biological response when they come across with biological molecules. For this reason complexes of metals with biological molecules are of key importance in biomedical applications. For instance, cisplatin is very rare in nature, but it is effective gainst cancer treatment in the form of complex with platinum. These have lead to great interest for the discovery of metal based new anticancer drugs [36]. Polymers that show response against external environmental stress have great importance in the field of medicine specifically for preparing drugs. These polymers perform task in delivering of drugs, cell adhesion, control function of enzyme and expression of genes [37]. Polysaccharides of sulphate have a great importance in many physiological processes such as coagulation processes, transmission of virus, and antioxidant activities. These synthetic sulphated polymers have many applications in the field of biomedical [38]. Metallic nanoparticles (MNPs) possess many physical and chemical properties which have been applied in many biomedical areas. These nanoparticles created an attention for the scientists to develop useful processes for the production of MNPs. Plants and microbes synthesize biological MNPs. Scientists have described the uptake of nanoparticles in cells, biocompatibility, cytotoxicity, biomedical applications and the interaction of MNPs with target cells [39].

During the nanoparticles synthesis process the two nanoparticles show different chelation process. The two hybrid nanoparticles (Cu0 AuNPs; L1@Cu^{2+}AuNPs) were studied by considering their physical and chemical properties by applying analytical techniques such as UV-vis spectroscopy, electron transmission microscopy, Raman and dynamic light scattering. Both systems exhibited good morphological and chemical properties at pH 4 during the period of 98 hours. Murine embryonic stem cells were used in the in vitro experimental conditions to monitor the effects of nanoparticles in these cells. The comparative study between Cu^0 AuNPs and L1@Cu^{2+}AuNPs highlights that copper chelated in its +2 oxidation state in the NPs is more functional for biological application [40]. Biopolymeric polyhydroxyalkanoates (PHAs) are fabricated and stored by microbes under unbalanced growth environments, commonly by a huge genera of bacteria. Microbially engineered PHAs have a great interest all over the world having many applications in biomedical field as biopolymeric biomaterials. Because of non-hazardous disintegration products, preferred surface alterations, inherent biocompatibility, modifiable mechanical properties, cultivation support for cells, adhesion devoid of carcinogenic impacts, and controllable biodegradability, the PHAs like poly-3-hydroxybutyrate, 3-hydroxybutyrate and 3-hydroxyvalerate co-polymers, 3-hydroxybutyrate and 4-hydroxybutyrate co-polymers, etc., are available for various medical applications [41]. Polymers and their elements have many biomedical applications such as regeneration of hard tissue, healing of wound, artificial skin, antibacterial oxygenators, and drug delivery carriers. Natural as well as synthetic polymers are used for biological, medical applications and have many benefits and a few limitations. Microarray technique helps in fast screening of valuable polymeric materials for biomedical applications and 3D printing helps in fabricating having desirable pores to minimize the architecture of natural tissues [42].

Tissue engineering and biomaterial science plays an important role for the development of regenerative medicine. Stem cells (SCs) are a distinct types of cells having the ability to self-renewal and reconstructing damaged tissues. Modern material technologies have many applications in biomedical field. Biomaterials, such as ceramics and metals, are already used as implants to replace or improve the functions of the damaged tissue or organ [43]. Based on synthetic biology, biomedical tattoo was prepared using engineered cells that would detect long-term blood calcium concentration, detect onset of mild hypercalcemia, and respond via subcutaneous accumulation of the black pigment melanin to form a visible tattoo. For this purpose, the cells were designed ectopically expressed calcium-sensing receptor rewired to a synthetic signaling cascade that activates expression of transgenic tyrosinase, which produces melanin in response to persistently increased blood Ca^{2+}. It was found that the melanin-generated color change produced by this biomedical tattoo could be detected with the naked eye and optically quantified. The system was validated in wild-type mice bearing subcutaneously implanted encapsulated engineered cells. All animals treated with hypercalcemic breast and colon adenocarcinoma cells produced tattoos, whilst there were no tattoos appearance in animals inoculated with normal calcemic tumor cells. All tumor-containing animals remained asymptomatic throughout the 38-day experimental period [44]. Green biobased polymeric membranes are highly emerging as materials of choice for many biomedical applications. As we know that in these membranes cases, the degradation is essential under physiological pH conditions, the working of membrane with sericin has many applications in bone marrow regeneration [45]. Many new technologies have been devised for drug delivery process that play an important role in the field of biomedical, the drug delivery process takes place from natural activatable materials like zymogens, membrane proteins, and metabolites, whereas stimuli initiate transformations that are necessary for cargo release, prodrug activation, or selective transport [46].

Polymersomes are structurally same as of liposomes, these structures are closed by a lipid bilayer membrane arises from amphiphilic copolymers. Polymersomes are considered to explain the structure and properties of cellular membranes and viral capsids. Excellent strength and stability, chemical versatility for tunable membrane properties and surface functionalization make polymersomes attractive candidates for drug delivery, diagnostic imaging, nanoreactor vessels, and artificial organelles. Furthermore, biomimetic techniques, stimuli-responsive polymersomes that can recognize various external physical or internal biological environmental stimuli and conduct “on demand” release in dose-, spatial-, and temporal-controlled fashions have been widely developed [47]. Synthetic biology is applied to medicine, biotechnology and biological research, therefore there is need to produce yeast, plants and mammalian cells through genetic engineering. Regulation of Eukaryotic genomes is carried out through biochemical and biophysical states of chromatin, which plays an important part in different challenges and opportunities, over applications in bacteria. Synthetic techniques such as ‘epigenome editing’, have allowed the direct and functional dissection of many features of physiological chromatin regulation. These studies are very necessary for biomedical and biotechnological engineering applications that could take advantage of the distinct combinatorial and spatiotemporal layers of chromatin regulation to produce synthetic organisms or organ systems that never happened in the past [48]. Dendrimers have distinct characteristics that make them special for use in biomedical applications. Now a days, by statistical analysis and changing of peripheral groups many types of multimodal dendritic structures have been synthesized. The development in the field of biochemistry and chemical biology, chemoselective methods are developed for ligation, through this ligation it is possible to synthesize and draw structures and apply in nanomedicine [49]. At the molecular level, mechanisms of diseases are being solved. Fluorogenic probes are applied for taking internal images of dienophiles [31].

5. SYNTHETIC BIOLOGY AND BIOENERGY

Energy security is considered one of the most challenges to the world today. The success from recent effort to obtain affordable, renewable and environmental friendly energy from solar panels or transgenic plants has been limited. Synthetic biology approach is considered a primer approach to the development of cost effective bio-based energy. SB contribution to bioenergy production involves two fronts: 1) improvement of existing biofuel production plants through protein engineering and biofuel producing metabolic pathway engineering to enhance biofuels production and 2) creation of new cell factory that are able to generate energy from both traditional and non-traditional feedstock through either strain development or importing genes encoding for biofuel production enzymes into a suitable host. SB employed state of art tools such as genome editing, and transgenic expression systems for biofuel production from lignocellulose, oil, and soluble sugars [50, 51].

Yeasts are considered an efficient biofuel producing agent that's has several advantages over its competing bacteria. Numerous synthetic biology studies have been carried out on yeast model *Saccharomyces cerevisiae* to engineer it for improved ethanol and fatty acids production. These studies have focused on investigation of regulation of gene expression at both transcriptional and post-translational level of biofuel producing genes. SB has resulted in identification of genes of cellulosic sugar utilization, and enhanced biofuel production from biomass. These studies have been well reviewed recently by [52, 53] and which are beyond the scope of this issue. Moreover, SB is broadening its investigation into nonconventional systems like *Hansenula polymorpha*, *Kluyveromyces lactis*, *Pichia pastoris*, and *Yarrowia lipolytica* which have excellent traits such as high ethanol tolerance, thermal stability, genetic diversity and tolerance to various inhibitors. Synthetic biology studies which have been carried out on nonconventional yeast for the purpose of enhancing biofuel production have also been reviewed by [53]. Other crucial microorganisms are microalgae which have emerged as potential alternate feedstock for biofuel production and numerous chemicals. Synthetic biology has been applied to microalgae for optimal biofuel productions and has been reviewed by [54, 55].

6. SYNTHETIC BIOLOGY AND DRUG DEVELOPMENT

Nature has huge reservoirs of bioactive compounds which are used by human to fight diseases since thousands of years. Human has also learned to isolate, characterize and even to synthesize these compounds in laboratories which considered a laborious and time consuming procedure. These bioactives are produced in small quantities and large-scale production from original sources is not efficient and unprofitable [10]. Genome sequencing has enabled us to acquire genetic information about metabolic pathways synthesizing these compounds and to produce them in pharmaceutical scales through genetic engineering which itself considered insufficient for number of reasons including those feedback mechanisms and organization of individual genes in time and space.

Synthetic biology is considered the best alternative approach to eliminate these limitations and produces these secondary metabolites in pharmaceutical scales [56]. Synthetic biology in this respect involves characterization of individual modules and recombines them in an artificial and predictable network. The application of SB in drug discovery and development is encouraged by 1) cheap DNA synthesis to produce the genetic element/circuit even entire genome for production of bioactive compounds, 2) Rapid progress in DNA sequencing and drop in the cost of sequencing which provide significant amount of genetic information about the biosynthesis of bioactive and their regulations, 3) development of well characterized genetics modules that are used to create new function [57]. Thus it is believed that SB will be employed to drug discovery and development in the present century what organic chemistry has been in the 20th century to pharmaceutical industry. SB incorporates engineering principles to living cells to produce biofactory. The first application of SB is creating new chemical scaffolds that have the characteristics of therapeutic agents derived from natural sources with desirable pharmacological properties [58, 59]. SB application in pharmaceutical production is artemisinin, an anti-malarial drug produced from sweet wormwood tree, by genetically engineering of yeast through introducing of six genes involved in biosynthesis of artemisinin precursors which can be developed into final medication. This approach is cheap and fast and is currently contributing about 35% of world's artemisinin supply [60]. Another example is hydrocodone, a painkiller produced from opium poppy, through introducing 23 non-native genes into the yeast genome making a strain capable of producing hydrocodone [6]. It is expected that SB will also contribute to the discovery of new antibiotics that will help us to fight the infectious disease particularly with rapid exploration of human microbiomes and finding that these microbes produce huge volume of secondary metabolites, majority of which are with antibiotics function. This represents an excellent place area to study new drug candidate and their metabolic biosynthesis and to apply the principles of SB to produce these bioactives in a commercial scales (Fig. 2) [59].

It should be noted that the application of SB in drug discovery and development is not limited to pharmaceutical production rather is involved in other steps of drug discovery and development. For example, the genetic circuits designed by SB are used in 1) drug discovery, 2) screening, 3) drug mechanisms of actions; 4) validation of drug target and drug delivery. More interestingly, SB is used in construction of diseases models with the help of optogenetics to decipher disease mechanisms such as cancer and neuronal diseases. Moreover, it is expected that bacteria will not only be used for production of pharmaceutics but also used for in site delivery of drug to targeted tissues [62].

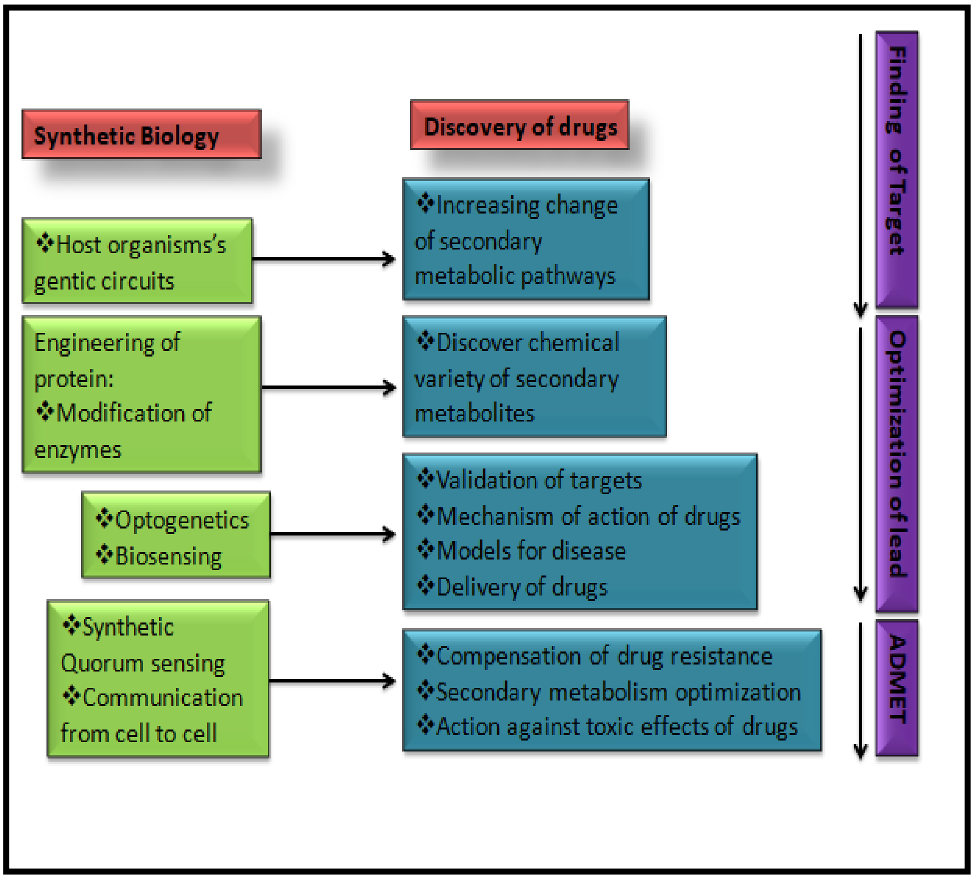

Fig. 2. The role of synthetic biology in different steps of drug discovery and developments [59].

7. SYNTHETIC BIOLOGY AND AGRICULTURE

Synthetic biology is considered to have least contributed to agriculture compared to microbial and biomedical sciences, however, it is expected to contribute significantly in the near future in crop development and bioproduction in plants. Plant synthetic biology combines engineering principles and plant biology science to design new production devices. Number of pioneering synthetic biology studies in plants has been published in the production of synthetic sensors and synthetic metabolic pathways [63]. Synthetic sensors are constructed to be controlled at the transcriptional or post-translation level when introduce to the engineered organisms and allow cells to report the presence of internal or external stimuli. The best examples of post translationally controlled sensor is for monitoring auxin-induced plant indole-3-acetic acid (IAA) degradation in yeast [64].

Addressing the challenges associated with synthetic biology application in agricultural sciences may assist in advancing the application of SB to agriculture. Application of SB to agriculture may be hampered because the industry is dominated by higher plant and polyploidy genomes are employed and lack of adequate tools harness the delivery of outcome soon. Hopefully, synthetic biology along with new genome designing and synthesis tools, and the most efficient tools such as CRISPR/Cas9 will enhance the opportunities in development of new cultivar, breeds, biosensors engineering, synthetic speciation, microbial metabolic engineering, mammalian multiplexed CRISPR, novel anti-microbials, all of which will deliver transformative changes to agriculture in longer term [65-67].

7.1 Applications of synthetic biology in agriculture

In the recent years, many advancements in the field of life sciences such as, discovery of DNA, genetic code, recombinant DNA technology, human genome mapping have played an important role in synthetic biology. Synthetic biology prepares living machines from chemical entities by employing same techniques as electrical engineers use to construct the chips for computers. It leads to automated synthesis of DNA molecules and their assembly into genes and microbial genomes as well as construction of functional genetic circuits and metabolic pathways for practical purposes. Apart these, it creates bioengineered microorganisms that may produce pharmaceuticals, can detect harmful chemicals, breakdown the pollutants, repair defective genes, destroy cancer cells, and produce hydrogen for petroleum economy. So, synthetic biology is the engineering field which constructs simple biological systems that offers scientists a very easy way to understand the complexity of genes and biomolecules that are necessary to carry out different life processes [68]. With the increase in demand for healthy food, there is urgent need for the production of anti-pests and anti-plant diseases products. There is an increasing demand for synthetic pesticides, but their plenty of usage is the main cause of environmental pollution as well as human health problems. So, it is urgent need to produce the drugs that are safer and can replace the already present drugs. Biological control is the basic need in this era to control pests and plant diseases which emerge as a result of usage of chemical pesticides. More than half (52%) of the researchers thought that synthetic biology should be regulated by the federal government, whereas 36% thought that voluntary guidelines developed jointly by industry and government could provide adequate oversight. One researcher comments about synthetic biology are: I feel [that federal regulation] is the best approach, because I don’t agree with banning [synthetic biology]. Technology should advance, and in order to advance… there’s risks with it. Anything that we have today comes at a risk, but… I think of the four [options provided], the federal government would be the best. And I guess it would be more in the spotlight than just some private company and its investors. He continued his explanation by referring to the broad principle of political accountability: “At least…, technically, we choose the government, so if they screw up, we can vote them out of office. And with all of the people on television discussing everything, it’s probably harder for the government to whitewash an issue as opposed to a private company [69]. Bacilli bacteria produce a number of biopesticides that have been used from the ancient times. They are used in agricultural biotechnology and their products are applied as microbial pesticides and fertilizers [70]. By adding organic matter to the soil increases bacterivorous and fungivorous in soil and it decreases parasitic nematodes [71]. Because organic matter increases soil fertility and it acts as food for the parasites in the soil. Plants and microbial parasites have a link between them like symbiosis [71]. Nematodes that are present in soil, they obtain high content of carbon and nitrogen from the soil and prepare their food, whereas nitrogen is being released from the soil as a waste product. When bacterial population is at the top in soil then nematodes can release amino acids in very high amounts. But when bacterial population declines, the nematodes suffer starvation and breakdown of proteins to recover need of energy, this leads to high amounts of ammonium excretion from nematodes [72]. Amount of nitrogen is an important factor to measure the microbial activity and at a time rate of breakdown [73]. Apart from, as we know that organic matter leads to increase in soil fertility, it also decreases the diseases of plants by the addition of antagonistic microbes. The crops grown by the use of synthetic nitrogen fertilizer were lighter than those grown by using pelleted chicken manure [74]. Chemical companies have spent a lot of money for the production of pesticides against insects, diseases of plants weeds and crops of agriculture. To control the diseases of plants, companies are preparing different types of fungicides having best performance to control biological methods. Now a days, biocontrol project is running in which baculoviruses have been used to control the insects. These are genetically modified viruses to kill the insects more faster than wild type. These are more safe and beneficial for insects as well as mammals as compared to synthetic pesticides. EPA is trying for synthetic pesticides to control diseases like, seedling disease, root rot and post harvest disease [75]. Allelopathy is a phenomenon in nature that is occurring in ecosystems and it creates interference between organisms that can be used for the management of weeds, insect pests and different types of diseases in field crops. For natural pest management, it may be employed by rotation, by using cover crops, mulching and extracts of different plants. So this process may effectively control pests and weeds. Different mixtures of allelopathic water extracts are more effective as compared to only one plant extract. Minimum amount of herbicide applications are more effective to eliminate the resistance against herbicides in weed ecotypes. Thus, allelopathy is an environmentally friendly to control pesticides in agriculture [76].

Carotenoids show a great deal of variety of color pigments or their related compounds that are essential for the process of photosynthesis, apart from, they also respond to biotic and abiotic factors in the environment and control plant structure. Containing all these qualities these pigments are more useful in the field of synthetic biology to change the form of plant and to fulfill the requirement of food and to apply these in agriculture and industries [77]. Plants can play a great role by attraction of herbivore predators with herbivore-induced volatiles (HIPVs). Entomopathogenic nematodes (EPNs) effect and kill insects with the help of symbiotic bacteria which may act as a biological control function in agro-ecosystems. EPNs are known to respond to herbivore-induced plant volatiles (HIPVs) that act as attractant cues increasing nematodes numbers in response to herbivores and as a result increasing mortality of root pests [78].

Genes may also be important for the control of agricultural insect pests, pests of animals (New World screwworm), plants (spotted wing Drosophila, diamondback moth, Bemisia tabaci whitefly), or stored grains (red flour beetle). The classical method in agriculture is developed to decrease insect pest populations by the use of insecticides and other farming practices [79]. CRISPR-Cas9 is a technology that is used to improve the growth of crops. Old methods for the breeding of plants are not enough to meet the increasing demands of food for the societies due to other environmental challenges. CRISPR has eliminated the hurdles for genome editing and could improve plant breeding [80]. The introduction of secondary metabolites in signaling between plants and other organisms has a great role in good crop production [81]. Worldwide increase in population and change of environment creates a challenge for the production of crop. Plants have association with microorganisms that promote the growth of plants, to support nutrition and food for plant, and kills the pathogens of plants. The relationship between plants and microorganisms play an important role to improve the agricultural production. Microscale information technology plays an important role for microorganisms applications under natural conditions [82].

Many unique features of cyanobacteria for example: photosynthesis in the presence of oxygen, greater production of biomass, growth on fertile land and varieties of water resources whether it is contaminated or polluted water, production of useful by-products and bio-fuels, they increase the fertility of soil and reduce green house gas emissions, are collectively an important bio-resource for better development of crops [83]. Integrated Pest Management (IPM) is an important factor and another technique for synthetic pesticides having has special importance for a wide variety of environments and human health. Productivity outcomes has been analyzed in Asia and Africa. Nonetheless, policy support for IPM is relatively rare, counter-interventions from pesticide industry is common, and the IPM challenge never done as pests, diseases and weeds evolve and move [84]. To investigate the result of silver nanoparticles (Ag-NPs) in a sludge-amended soil cultivated with monocot (Wheat) and dicot (Rape) crop species. A pot experiment was performed with sludges produced in a pilot wastewater treatment plant containing realistic Ag concentrations (18 and 400 mg kg^{-1} , 14 mg kg^{-1} for the control). X-ray absorption spectroscopy (XAS) showed that Ag_2S was the main species in the sludge and amended soil before and after plant culture [85].

Scientists are trying to reduce nitrate contamination in agricultural fields by applying synthetic fertilizers in agricultural fields and to retain the nitrogen capacity in the soils [86]. In the future, the majority of synthetic biological systems will be engineered by transferring small circuits of genes into a bacterial host, that will limit the risk [68].

8. SYNTHETIC BIOLOGY: CHALLENGES

8. 1 Technical based challenges

Although synthetic biology power is beyond imagination e.g., cells may be programmed to deliver drugs, detect toxin and produce biofuel, technically synthetic biology is challenged by number of difficulties. For example, many biological component such as genes and protein lack clear description about their function and whether they work under laboratory conditions in the same way in the living cells. Similarly, the construction of genetics circuit is greatly difficult and unpredictable and researcher has to test those variant separately to find which one sufficiently produces reliable outcomes. Another technical challenge is that the biological circuit is largely complicated and time consuming as the actual function of combined component might not be as predicted, and synthetic biology has tied up with many ups and downs processes which increase in the cost [87]. Synthetic biology in eukaryotic cells is challenged by 1) poor success of transformation into both cells and organisms, 2) slow growth which usually takes months to integrate gene systems into their genomes, 3) precision of DNA integration site which can now be improved by CRISPR-based tools that improve site specific integration into reliable chromosomal regions and 4) Chromatin complexity added by alternative splicing, post-translation modifications in eukaryotes [88, 89].

8.2 Ethics based challenges

Synthetic biology is faced by strong opposition led by Erosion, Technology, and Concentration (ETC) group which called for global moratorium on developments in synthetic biology. For example, on 2008 ETC published a critical report entitled "Extreme Genetic Engineering." Subsequently, on March 2012, the statement "principle of the oversight of synthetic biology" was issued by more than 100 environmental and civil society organizations which called for worldwide monitoring of commercial use of synthetic organisms until more robust regulations and rigorous biosafety measures are established. Thus societal issues such ethics, safety, security and public perception related to Synthetic biology have been discussed. The Woodrow Wilson and Hastings center are prestigious institutes of bioethics published a report in 2009 indicating that ethical concerns in synthetic biology have received scant attention. As of 2010 the national Academies committee of science, technology and law released a report on bioethical issues titled "New Directions: The Ethics of Synthetic Biology and Emerging Technologies" to the President calling for enhanced federal oversight in them emerging field of synthetic biology [90-92].

8.3 Safety based challenges

Synthetic biology also faced some safety-based challenges; the first and most crucial one is being that related to the side effect of synthetic organisms. Researchers have claimed that the synthetic organisms can be used for performing some job e.g., decontamination of soil or water but these organisms may have unwanted or negative side effects of environment and hence on ecosystems balance. The second risk that the synthetic organisms may transfer genes to the natural organism through gene transfer mechanisms. The third risk that the self-replication of synthetic cells may become out of our control and results in great disasters especially those mechanisms of pathogenic organisms is not fully understood, and it is difficult to determine which agent is hazardous [93, 94]. Synthetic organisms may be used by bioterrorism actions thus need to be highly under oversight. Moreover, genetic circuits function is unpredictable and unwanted side effect should be anticipated and safety actions should be taken into consideration. The international agencies such UN, Chemical Weapons Conventions, and Biological Weapons Conventions should work together impose regulation on DNA synthesis and monitor access to database to recognize suspicious DNA orders in USA and France. The safety issues related to synthetic biology should be discussed through education, conferences, and seminars. The lessons should be learned from documented cases of virus leakage from laboratories, such as smallpox in the United Kingdom (1978), SARS in Singapore (2003) and China (2004). It is important to protect human, animal and the ecosystems from such accidental release of synthetic biology agents [90, 95, 96].

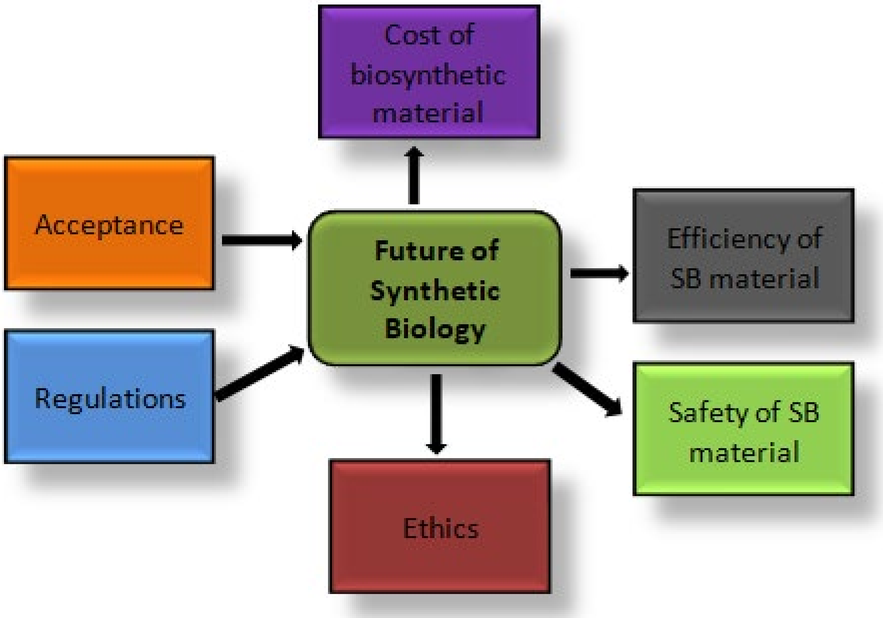

Fig. 3. The parameters affecting the Synthetic Biology future [90].

9. CONCLUSIONS

Synthetic biology applies engineering principles to construct new bio-based biologicals, devices and systems that perform functions do not present in nature or to redesign the existing system to perform specific tasks. Synthetic biology has tremendous potential applications in biomedical science, drug discovery and development, food industry, biofuel production, and agriculture. However, most of Synthetic biology applications are at their infancy. Moreover, synthetic biology is being challenged by numerous issues related ethics, safety and biosecurity and addition to those related to technical aspect. The researchers need to work hard and wise to overcome these challenges.

CONFLICT OF INTEREST

No conflict of interest

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