Prospect of Bioremediation Using Synthetic Biology Approach in Indonesia

Rahmat Azhari Kemal¹, Annisa Novianti², Ilma F. Ma'ru³, Ika A. Rini¹, Ruli Fatmawati³

¹Department of Biotechnology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, Indonesia
²Department of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, Indonesia
³Department of Microbiology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, Indonesia

Corresponding author: rahmat.rakemal@s.itb.ac.id

Abstract

Cellular systems can be engineered by synthetic biology approach to make a controllable bioremediation system. Several teams competing in the International Genetically Engineered Machine (iGEM) competition have utilized the potency of synthetic biology for bioremediation. This review highlighted several ideas. One of them is the designing a whole-cell biocatalyst that can degrade PET component in plastic waste. Rice blast (Magnaporthe grisea) produces cutinase, thus its gene encoding cutinase can be used as substitute in the system. Regulation of the application is also discussed. It is hoped that research and implementation of bioremediation using synthetic biology in Indonesia can be developed.

Keywords: Biodiversity; bioremediation; iGEM; Indonesia; synthetic biology

1. Introduction

Molecular biotechnology allows researchers to transfer particular units of genetic information between organisms to improve their values [1]. Gene is expressed as a response to accumulated specific signal and this system is used by the researchers to control which chemicals or materials are produced by the cells. Production system may be enhanced or designed for new applications by performing computation in a living cell for example to control gene expression by timing or for certain conditions [2]. This controllability serves as one of the foundations of a new emerging field of synthetic biology.

Engineering discipline of biology called synthetic biology, unlike biotechnology field, needs standard parts that can be put together using bioinformatic and simulation tools to build circuits that will introduce or modify biological functions. Existing cells and organisms are modified so they work as cell factories. This project involves the use of standardized parts (genes, proteins, circuits). Group of European experts defined synthetic biology as the engineering of biology which is the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature [3]. Consequently, synthetic biology is developed by a program and a chassis. A program is described as the conceptual extension of the genetic program, while chassis is the conceptual extension of the living cell [4]. The choice of regulators and switch to change gene expression will influence the circuit dynamics of genetic program [2].

The International Genetically Engineered Machine (iGEM) Foundation is an independent, non-profit organization dedicated to education and competition in synthetic biology. It began in January 2003 as an independent study course at the Massachusetts Institute of Technology (MIT) where students developed biological devices. The following year this course became an annual summer competition [5]. The competition is held by creating genetically engineered systems using standard biological parts called BioBricks which is over 20,000 in numbers. Every year each team raises new global challenges and their solutions. The subject could be something new or improved from the previous project done by the other team. Hence, every team conveys new information about particular biological parts. Therefore this competition is offering a novel solution based on synthetic biology approach.

There are various tracks for iGEM competition, one of them is environment. Many teams address the bioremediation application of synthetic biology. Cleaning up the contaminated environment, either by organic or inorganic matters, can be done by biological factors. The natural process of bioremediation is promising albeit possesses several drawbacks [6]. Long-time, specific, and limited application of bioremediation can be overcome by genetic engineering. The modularity of synthetic biology can overcome complexity in biological system [3], therefore will take the genetic engineering for bioremediation into a new level. Here we review several ideas raised by iGEM teams for bioremediation application.

2. Synthetic Biology for Bioremediation

Wastewater is one of major problems in many countries in the world, especially in Indonesia. Wastewater treatment is an essential process as it is important to avoid its negative impacts on environment. One advanced treatment is membrane bioreactor process (MBR). This process uses membrane coupled aerobic bioreactors and an activated sludge system with an integrated membrane. Cell immobilization to hollow fiber membrane bioreactor has
been showed to improve bioreactor design [7]. Microbial biofilm is one form of immobilization. It may also contain noncellular materials such as mineral crystals, corrosion particles, clay, or silt particles [8]. Porous silica immobilization of cells (biosilification) can be used for high quality thin film deposition. Therefore, Purdue iGEM team 2012 project tried to develop a device in Escherichia coli that will induce the expression of adhesion proteins to facilitate biofilm formation and the production of silicatein alpha, a silica binding protein (SBP) to form biofilm’s silica coat [9]. In wastewater treatment, water is run over the biofilm, and microorganisms absorb and digest undesirable compounds. Exterior silica coat will help to prevent biofilm breakage as well as act as a mechanical filter to remove any large particles from the water [7]. In Indonesia, silicatein is can be found forming nano-structure silica called biosilica in sponge (Phylum Porifera) from Nias and Lombok seacoasts [10].

Synthetic biology can also address the waste problem. Waste treatment can be applied in poultry feather waste that is mainly composed by keratin protein. Traditional methods of keratin degradation rely on chemicals such as sulfides and limes which pose serious environmental and health concern [11]. Although keratin-degrading bacterial strains have been isolated from poultry waste nearly two decades ago and some keratin-degrading proteases (keratinases) have been identified, keratinase production in heterologous hosts faces challenge in the form of poor protease expression. Therefore, iGEM 2013 team from University of Chicago constructed B. subtilis constitutively expressing keratinase [12]. This project shows that utilization of synthetic biology could be supported by the availability of biodiversity as part of genetic module. One species capable of producing serin keratinase is B. licheniformis [13]. In Indonesia, B. licheniformis has been isolated from Bukit Kili hot springs in Solok, West Sumatra [14] and Tompaso Geothermal Springs, North Sulawesi [15], which have temperature more than 50°C. Therefore, the strain may provide a thermostable keratinase that can be applied in poultry waste treatment.

Plastic waste problem can also be solved using synthetic biology. ITB Indonesia team designed a system for plastic degradation, not only to degrade it but also to use it for growth, making it a zero waste system [16]. Their synthetic E. coli will be able to degrade polyethylene terephthalate (PET) plastic. The product of degradation will be ethylene glycol (EG) which is harmful to the bacteria. But by introducing a conversion system, they designed the bacteria to be able to utilize EG as energy source. The implementation of this system will help in solving plastic waste problem. Rice blast (Magnaporthe grisea) has 16 putative cutinases in its genome [17], thus enzymes can be studied for their prospect to substitute LC-cutinase used in the system.

Decontamination of sites is another focus of bioremediation. Contaminants can belong to organic or inorganic matter. The iGEM 2013 team from University of Sydney also used Xanthobacter autotrophicus to degrade low levels of dichloroethane (DCA), an organic compound, found in aquifers around Botany Bay, since this bacteria contain one pathways of particular interest. Conventional DCA treatment is both costly and time-consuming, involving pumping and heat-stripping groundwater. Therefore, they construct their own versions of two metabolic pathways of DCA biodegradation, and characterize their effectiveness in utilizing DCA as a sole carbon source for growth [18]. X. autotrophicus can also be used as bioremediation agent used for petroleum contaminated soil by using hydrocarbon as sole source of carbon [19]. This species is an indigenous bacteria found in the contaminated sites Sungai Lilin Jambi Pertamina Ltd which could degrade the petroleum oil sludge faster than the other indigenous bacteria (Pseudomonas pseudoalcaligenes and Bacillus megaterium) [20].

Contamination by inorganic matter such as heavy metal has become serious environmental problem, too. Several organism have been developed mechanism to protect the cell from heavy metal exposure. In order to survive prolonged exposure of undegradable heavy metal, bacteria develop few mechanisms such as cell surface binding, influx and efflux, accumulation, detoxification of heavy metal with protein called metallothionein [21]. Metallothionein is also used by UMBC iGEM team to remove the copper pollution in the water. With synthetic biology, E. coli is manipulated to produce metallothionein in their surface, increasing copper sequestering and avoiding toxic effect towards the cells. [22]. Heavy metals-resistant and metallothionein-producing bacteria have been isolated from natural resources in Indonesia, one of which is in a gold mine in the villa Pongkor, Bogor. Bacteria Brevundimonas sp. was shown to accumulate heavy metals, especially mercury in gold mining [21]. The bacteria can be genetic source for metallothionein for bioremediation application.

3. Regulation of Synthetic Biology-Based Bioremediation

The arrival and development of all biotechnology subjects in Indonesia can not be liberated from the safety aspects. The government chooses to take the precautionary approach to avoid the potential risks. On May 21, 2000, Indonesia signed the Cartagena Protocol on Biosafety. The Cartagena Protocol is the Protocol for regulating safe transfer, handling and utilization of LMOs (Living Modified Organisms) [23]. The objective of the protocol is to ensure a high level of protection regarding the safe transfer, handling and utilization of LMOs resulting from modern biotechnology which may potentially have harmful effects on the conservation and sustainable utilization of biodiversity, with a special emphasis on human health and a specific concern with trans-boundary movement [24]. Since generally the distinct between synthetic biology and biotechnology are rely on technologies or issues of how they are controlled [25], therefore the Cartagena Protocol is only valid for biotechnology field.

In 2011, The International Civil Society Working Group on Synthetic Biology suggested to the Convention on Biological Diversity’s Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA) on the
Potential Impacts of Synthetic Biology on the Conservation and Sustainable Use of Biodiversity that; (1) The Cartagena Protocol does not sufficiently cover synthetic biology and its potential impacts on biodiversity; (2) The Cartagena Protocol does not cover the virtual (digital) transfer of LMOs; (3) The Cartagena Protocol does not cover the transfer of constituent parts of an LMO that can be readily assembled, and (4) The Cartagena Protocol, so far, allows for the import of synthetic organisms into contained use without analyzing and adapting the containment standard [26]. They suggested to; (1) Consider extending requirements for advance informed agreement and risk assessment procedures to synthetic genetic parts in order to cover gaps that otherwise permit evasion of the rules agreed under the protocols; (2) Consider excluding from the ‘contained use’ provisions, synthetic genetic parts and living modified organisms produced by synthetic biology, in order to address the new containment challenges they pose - at least until effective containment methods can be demonstrated; and (3) Consider the case in which an agent imports an LMO into containment (without obtaining advance informed agreement) and subsequently seeks to take it outside containment, that such an agent be then required to obtain an approval from the domestic regulator based on a risk assessment process [25]. Therefore, if Indonesia is to make regulation regarding synthetic biology application in bioremediation, it needs to address not only Cartagena Protocol, but also the recommendations from The International Civil Society Working Group on Synthetic Biology.

4. Conclusion

Synthetic biology approach in bioremediation is full of potential. Ideas from iGEM teams addressing the problem can be adapted by Indonesia using the natural and genetic resources available. Regulations about the application must adhere to Cartagena Protocol and put the recommendations made by The International Civil Society Working Group on Synthetic Biology into account.

References