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Advances in Design and Use of Microbial Production Systems: A Workshop for the BWC Community

Workshop Summary

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Prepared under the auspices of the IAP Biosecurity Working Group

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CONVENING ORGANIZATION AND SPONSORS

The workshop and summary were produced under the auspices of the Biosecurity Working Group of IAP: The Global Network of Science Academies. IAP, formerly known as the InterAcademy Panel on International Issues, is a network of 111 of the world's academies of sciences. Its primary goal is to help member academies work together to advise citizens and public officials on the scientific aspects of critical global issues. The IAP Biosecurity Working Group was established in 2004 to undertake IAP's work at the intersection of biosciences and security. The Working Group now includes the academies of Australia, China, Cuba, Egypt, India, Nigeria, Pakistan, Poland (chair), Russia, United States, and United Kingdom and concentrates on two issues: a) education about dual-use issues in the context of responsible conduct of science, and b) implications of trends in science and technology (S&T) for the operation of the Biological Weapons Convention (BWC) and other nonproliferation treaties.

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SUMMARY

A workshop was held in August 2015 under the auspices of the IAP Biosecurity Working Group to discuss trends in microbial and biologically-based (bio-based) production. The meeting examined how the design and scale-up of such systems is changing the nature of producing biological and chemical products, what factors are driving this expansion, and what implications these developments may have for the implementation of the Biological Weapons Convention (BWC).

Microbial systems can be used to produce therapeutic proteins, as well as chemical molecules such as drugs and biofuels. Microbial production of high value and feedstock chemicals is becoming an increasingly attractive option as factors involved in global supply and demand have changed in response to scientific and technical developments, current economic conditions, and national biotechnology investments. The possible applications of bio-based production continue to expand, enabled by advances in the ability to manipulate genes and metabolic pathways through synthetic biology. The meeting explored examples of companies at the forefront of these trends, including in the creation of design tools and platform technologies that are accelerating the progress of microbial and bio-based production and increasing its reliability, fidelity, and simplicity.

INTRODUCTION AND EXAMPLES IN ACTION

The bioeconomy is built upon advances in the application of science and technology to living organisms and a number of factors are driving a growing interest in it, including availability of feedstock resources, environmental considerations, and financial investments and incentives. The first session of the workshop provided an overview of bio-based production and explored changes to the chemical and biotechnology industries through examples of activities in several countries. As the presentations highlighted, industry is global in scope and speakers noted a number of international partnerships in the development of bio-based production systems. Biological industry is not a monolithic entity, however. Drivers and challenges vary in different sectors, for example for higher-margin, smaller batch specialty products versus bulk chemicals such as biofuels.

Industrial use of bio-based production systems: drivers and challenges

Detlef Männig, Evonik Industries

The bioeconomy, which encompasses the production of renewable biological resources and the conversion of these resources and waste streams into value-added products including food, feed, other industrial and medical products, and energy, makes up a major portion of economic activity across the globe, promoting a more resource-efficient and sustainable economy. Evonik, a German specialty chemicals company, is a prime example of the integration of biotechnology into a traditional chemical company. Advances in metabolic engineering, fermentation, and biocatalysis are allowing Evonik to shift production of a number of specialty chemicals, polymers, and amino acids to bio-based methods (Figure 1). Evonik draws on both in-house research and development (R&D) and venture capital investments to advance its bio-based portfolio, with 10% of its internal R&D budget and 1/3 of its total venture capital budget going towards biotechnology research.

Many factors influence the development of the bioeconomy within companies and externally. Government and industry regulation can dramatically impact adoption and implementation – more than 20 countries have adopted national bioeconomy strategies, shaping prices and influencing investment decisions. Regulations based on renewable energy and sustainability also factor in pushing the bioeconomy forward. Other significant external drivers include energy costs (fertilizers, transportation, and fuel used to grow and harvest feedstocks used in bio-based production), the availability and reliability of raw materials, and new biotechnologies.

Within a company, a primary factor influencing the adoption of bio-based production is profitability. Customers may not be willing to pay a premium for bio-based products versus ones produced by conventional methods. Companies must demonstrate a clear advantage during life cycle analysis to justify a new production method, including economic and/or ecologic benefits. Maintaining quality, consistency, and year-round availability can also be a challenge when developing bio-based production methods compared to chemical synthesis. As with any new and developing technology, it may take years of research before a product or process becomes profitable, so companies must be willing to make long-term investment decisions and take on risk in order to reap benefits in the future. As expertise from a wider range of disciplines becomes increasingly relevant, companies also need to consider how to incorporate new areas of knowledge, tools, and career paths.

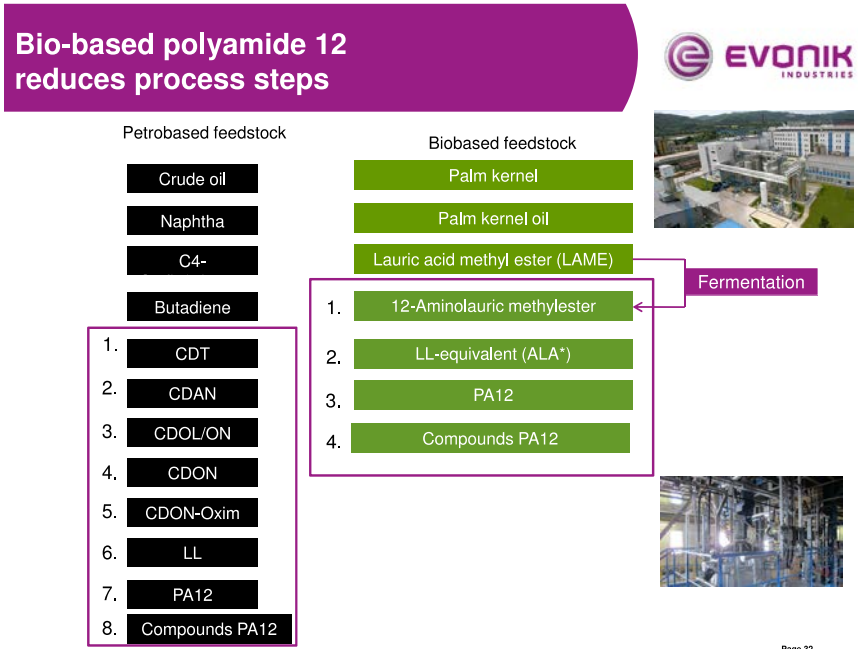

FIGURE 1 A biological feedstock streamlines the process for producing polyamide 12, which is used in packing materials, bags, and films. SOURCE: Presentation to the workshop by Detlef Männig, Evonik Industries, used with permission.

Developments in biofuels and green chemicals in Brazil

Alfred Szwarc, Brazilian Sugarcane Industry Association (UNICA)

Sucrose derived from sugarcane and other sources serves as an important feedstock material for the production of a number of industrial and commercial products. New processing techniques

are emerging that use sugarcane biomass for the production of fuels, cosmetics, biopolymers, and household products (Figure 2). Brazil is a leader in the application and utilization of bio-based energy sources and sugarcane bi-products harvested from biomass, i.e. trash leaves and tops from the sugarcane plants, and bagasse material remaining after sugar extraction, produce 3.3% of Brazilian electricity.

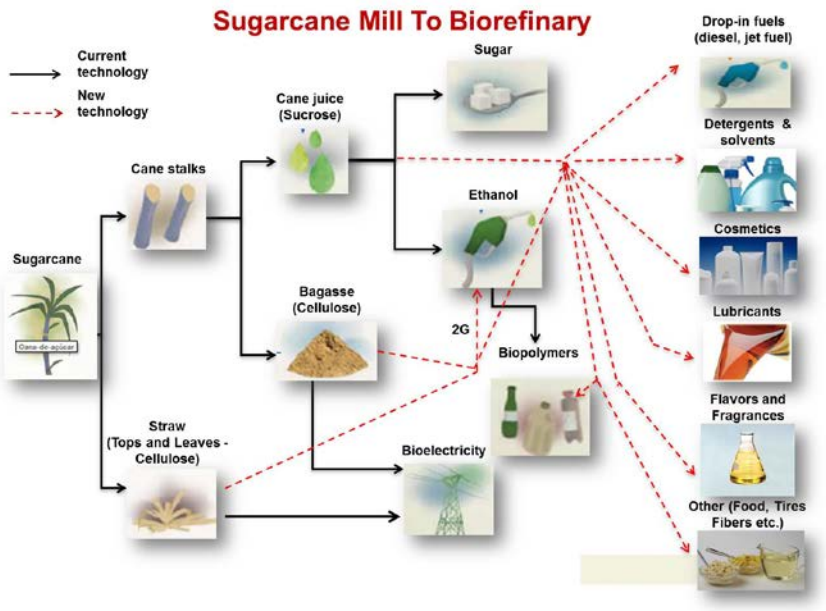

FIGURE 2 Sugarcane and sugarcane-derived materials can be used to produce a number of valuable commercial products and fuels. SOURCE: Presentation to the workshop by Alfred Szwarc, UNICA, used with permission.

A number of international partnerships are helping the Brazilian sugarcane industry take advantage of technologies such as new strains of genetically modified yeast and bacteria, and new enzymes. Through a partnership with Solazyme, for example, sugar is converted by microalgae to BioOil, used by cosmetics and personal care companies. Through a partnership with Amyris Biotechnologies, sugarcane juice is fermented by engineered microbes to produce drop-in bio-diesel and bio-jet fuel. In 2012 the Brazilian airline Azul flew a plane from Campinas to Rio de Janeiro on 50% bio-jet fuel, and in 2014 the Brazilian airline GOL flew a Boeing 737 on 10% bio-jet blend from Orlando, Florida to Sao Paulo.

The strong biotechnology sector in Brazil is aided by federal programs that promote biotechnology in the sugarcane industry. PAISS is a government program that provides low cost financing and grants for innovation and technology related to sugar cane, for example for 2nd generation ethanol production and sugarcane biomass utilization. BIOEN, through Sao Paulo State, provides grants for R&D. Additionally, in 2015 the import tariff was reduced from 14% to 2% for all enzyme-based products to be used in 2nd generation ethanol production or bio-derived chemical products.

Although there is significant institutional support for biotechnology in Brazil, low oil prices are currently limiting interest in renewable energy alternatives. For bio-fuel and other sugarcane-based energy sources to replace fossil fuels, market competitiveness must be demonstrated. As

stated in the previous presentation, consumers may not accept paying a premium for these technologies. In order to increase market competitiveness, further regulatory interventions to incentivize investment can be a helpful strategy – for example, a mandate on the minimum content of ethanol in gasoline.

Advancing the Malaysian bioeconomy through production of next generation oleochemicals
Muhammad Farish Kamaludin, Malaysian Biotechnology Corporation

A particular focus for Malaysian industry has been biomass conversion from the oil palm tree, which has the highest yield of plant oils per unit area and produces fruit for 20-25 years. The oil palm currently produces two types of oils from its fruit, crude palm oil and crude palm kernel oil, and there is potential for additional feedstock utilization from cellulosic sugar and lignin from palm fronds and empty fruit bunches, as well as bio-gas from palm oil mill effluent. These bio-feedstocks are generally converted to value-added chemicals via fermentation, enzymatic processing, catalysis, and/or thermochemical reactions (Figure 3). Additional R&D is being conducted to investigate other potential bio-feedstocks, including cassava, sugarcane, paddy, tea, and rubber, all native to ASEAN countries.

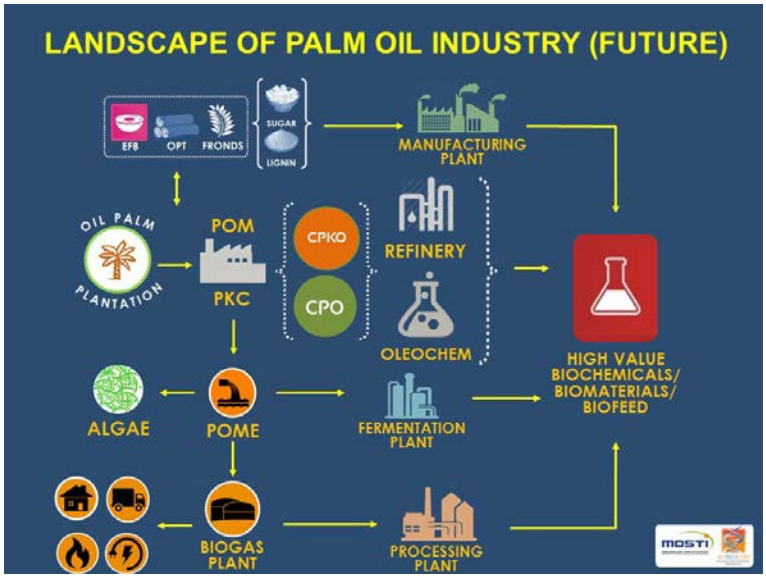

FIGURE 3 A number of pathways lead to the production of biochemicals and biomaterials from the oil palm tree. SOURCE: Presentation to the workshop by Muhammad Farish Kamaludin, Malaysian Biotechnology Corporation, used with permission.

Malaysia has significant biological resources as well as infrastructure and engineering expertise to support bio-based production, although challenges remain. In order to take advantage of developments in biotechnology and synthetic biology, including scale-up expertise, partnerships are a common strategy employed by companies in the Malaysian bioeconomy. The Malaysian government has also made significant investments in promoting the bioeconomy ecosystem including the establishment of dedicated industrial parks and infrastructure for biotechnology R&D. At Bio-Xcell in Johor, for example, companies are making dodecanedioic acid, adipic acid, and sebacic acid via fermentation, using glycerin as a fermentation feedstock in the production of lactic acid and bio-isoprene, and producing insulin and other pharmaceuticals via biomass

fermentation. Building shared facilities decreases the capital investments needed by companies to participate, promoting further development of these specialty chemical industries. In addition to infrastructure investments, the Malaysian government has introduced a variety of regulatory incentives for companies participating in the bioeconomy. Incentives include tax deductions for technology acquisition, import tax exemptions, industrial building allowances, and unrestricted employment of foreign scientists, allowing companies to recruit talent from around the world.

Advances in design and use of microbial production systems
Ryszard Slomski, Poznań University of Life Sciences, Polish Academy of Sciences

A number of advances in chemical and biological production are being explored in Poland, and the presentation provided information on several ongoing projects. The National Centre for Research and Development in Poland and the University of Life Sciences in Poznań, for example, are working together to develop a technique for the production of 2nd generation ethanol from the biomass of sorghum and miscanthus. Other collaborative research efforts include the development of cannabinoids with low THC content for cancer patient treatment. In addition, research is being undertaken to study the bio-conversion of glycerol to polyols and dicarboxylic acids and into 1,3-propanediol, a product used in materials such as polyurethanes, polyesters, and resins. Components of the metabolic pathway to convert glycerol to 1,3-propanediol were transferred from pathogenic bacteria such as *Klebsiella pneumoniae,* which are good natural producers of these molecules, into the nonpathogenic bacterium *Escherichia coli*, demonstrating the utility of synthetic biology to improve industrial biotechnology processes.

Such biotechnology advances in Poland and across the world have been accelerated by the rapid decrease in the price and time needed for genome sequencing, as well as a rapid increase in computing capability (Figure 4).

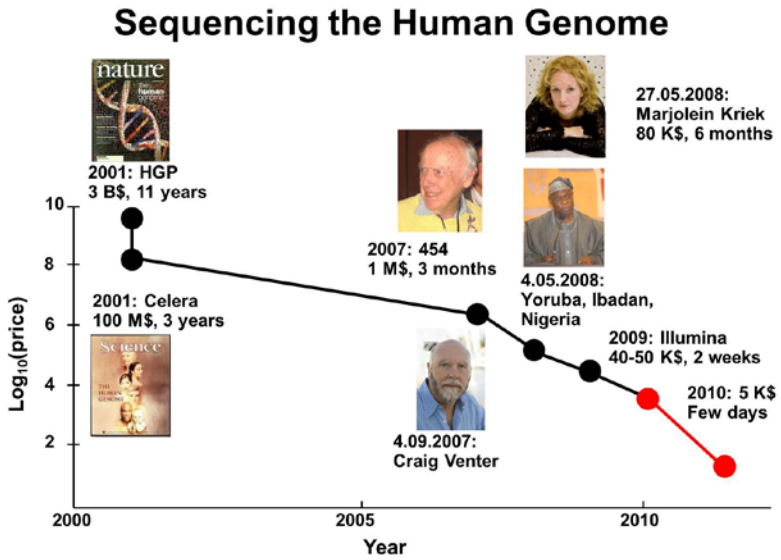

FIGURE 4 The cost and time to sequence genomic DNA has fallen dramatically since the turn of the 21st century, enabling a number of biotechnology advances. SOURCE: Presentation to the workshop by Ryszard Slomski, Poznań University of Life Sciences, used with permission.

STRATEGIES FOR REDUCING DESIGN AND DEVELOPMENT BARRIERS

Progress in a number of areas supports the growth of microbial and biologically-based production, including advances in fields such as synthetic biology and efforts to make biological design and development processes easier, faster, less expensive, and more reliable. The afternoon session provided examples of research institutes and companies that are tackling challenges such as improved design tools, rapid implementation and automation platforms, and the creation of special-purpose engineered organisms.

Developing an integrated design tool: Recent progress and remaining challenges
Markus Herrgard, Technical University of Denmark

The Novo Nordisk Foundation Center for Biosustainability (CFB) at the Technical University of Denmark is a non-profit center exploring the use of bacterial and yeast strains for the production of bulk and fine chemicals and food ingredients, and the use of mammalian cell lines for the production of protein-based therapeutics. To support translational efforts in microbial cell factory design and production, CFB utilizes a strategy called iLoop (Iterative Cell Factory Development Loop), in which specialized teams support different phases of product development: designing, building, testing, and analyzing the organisms involved.

The design of microorganisms can take place at a variety of biological levels – DNA, protein, pathways, organisms, communities, processes, and industries (Figure 5). At most levels, rational design is difficult because of the large numbers of possibilities to test. Achieving predictable control is also challenging for reasons including context-sensitivity of genetic "parts", inability to fully predict key aspects such as enzyme-substrate specificity, unanticipated side effects, variation in the way strains respond to changes in processing conditions, and others. Although empirical data are required for optimization, a key advantage of design tools is that they allow researchers to reduce the search space for screening new organisms with desired properties. Although investigators cannot predict whether or not a gene construct or engineered organism will behave as expected, by limiting the search space, they can avoid iterations that would not be worth pursuing.

Design complexity

Level	Length	Number of choices	Unit	Design space
DNA	50	4	Base pair	**10^30**
Protein	300	20	Amino acid	**10^390**
Pathway	15	5	Part	**10^6**
Organism	1000	3	Gene	**10^9**
Community	3	50	Organism	**10^23**
Process	10	5	Unit operation	**10^5**
Industry	1	10000	Chemical product	**10000**

FIGURE 5 There are a number of biological levels at which design can occur, from genes to industrial scale production. SOURCE: Presentation to the workshop by Markus Herrgard, Technical University of Denmark, used with permission.

A number of tools are increasingly available at all levels of design - examples include software programs to model the effects of gene modifications and databases to aid in predicting enzyme function based on sequence similarity to known proteins. Efforts are underway to integrate methodologies between levels. Design exchange standards are also emerging, allowing for improved data sharing and more rapid implementation of *in vivo* testing. The establishment of best practices and a systematic investigation of experimental validation methods will further aid in supporting the adoption of bio-based production.

Streamlining laboratory workflows and data analysis
Sean Ward, Synthace Ltd.

The bioeconomy continues to grow and industrial revenues to increase in sectors such as biofuels, food and agriculture, biologics feedstocks, and biochemicals. At the same time, the price per base of DNA sequencing and synthesis has fallen dramatically. However, the number of drugs produced per unit of R&D investment is falling – there is now less than 1 new drug produced per $1 billion of R&D funds. Dr. Ward referred to this as Erooms's Law (the inverse of Moore's Law).

To address these increasing R&D costs, Synthace is working to transform biological experimentation from an almost "artisanal" experience to a more uniform, automated process. Variables associated with a test organism and other experimental conditions can be broken into discreet operations. For example, an experiment may be divided into components such as feedstock, temperature, time, aeration, culture medium, and others. Factors associated with an experimental organism might be expressed in terms of the genetic expression cassette used in the study, locations of genomic integration, and chaperone molecules present. By separating experimental dimensions into discrete variables, an automated system can more systematically establish correlations among many factors being changed at once. Synthace manages this design and data collection through a software program called Antha, which allows researchers to select experimental conditions, repeat and modify previous runs, and keep an electronic log of results that can be analyzed to determine the sets of conditions that yielded desired outcomes. The experiments are carried out using automated robots, reducing user error. The Antha platform is hardware agnostic so that it is interoperable and transferrable among users at different labs. Scalability can also be included in the design of experiments, allowing for easier transfer from lab-scale to industrial-scale applications. By automating biology in this manner, Synthace seeks to decrease costs, increase experimental iterations, and enable data to be shared more effectively across multiple researchers, increasing the possibility of breakthrough discoveries (Figure 6).

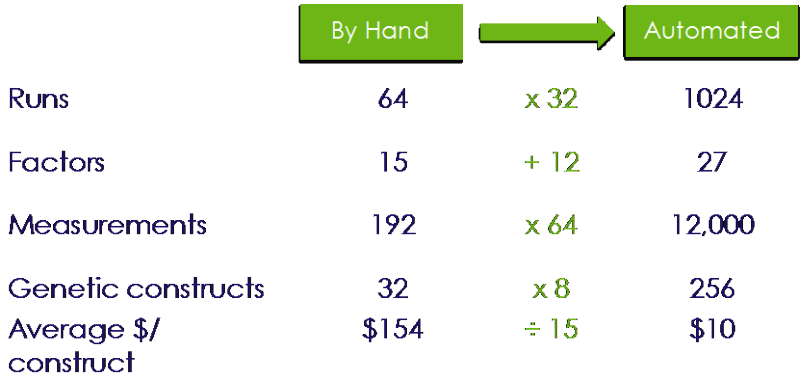

FIGURE 6 The use of software platforms to manage experimental design and the incorporation of control robotic handling increase the numbers of measurements that can be made and decrease the average costs to create a new genetic construct**.** SOURCE: Presentation to the workshop by Sean Ward, Synthace Ltd., used with permission.

Identifying disruptive technologies in biotechnology
Jason Kelly, Ginkgo BioWorks

The patent on recombinant DNA was awarded in 1974 and Dr. Kelly pointed to it as the first disruptive biotechnology. While there was early recognition of the significance of recombinant DNA technology, it was not possible to predict the many ways in which its value would be realized. Over time, major research programs and well-known companies became established to manipulate DNA in the development of protein therapeutics, agricultural products, industrial enzymes, diagnostics, oil cleanup strategies, vitamins, animal feed, brewing, dyes, and many other applications.

Dr. Kelly posed the question, is bioengineering a similar disruptive technology? The database of gene sequences is doubling every 18 months and costs are continuing to fall – suppliers can fabricate DNA sequences up to 5,000 base pairs in less than 90 days. Researchers are developing more standardized systems of biological parts and devices, making biology easier and more reproducible to manipulate. Ginkgo BioWorks is harnessing these technologies to create engineered microbes to be used in fermentation processes, turning feedstocks into high value chemicals and ingredients. As “the organism company” Ginkgo partners with others, for example with chemical companies, to design microbial strains for specific purposes and to integrate them into a company’s workflow. Ginkgo BioWorks has thus established itself as an organism foundry integrating software, robotics, and laboratory personnel with a vision of functioning like an organism manufacturing facility (Figure 7). A goal for this kind of centralized organism development resource is to expand the capabilities of companies interested in biotechnology and to further increase the opportunities for bio-based production of goods.

FIGURE 7 Ginkgo BioWorks combines personnel, software, and robotics into an "organism foundry" to facilitate the development of engineered microorganisms. Such specialized resources currently remain complex and expensive to replicate, however. SOURCE: Presentation to the workshop by Jason Kelly, Ginkgo BioWorks, used with permission.

DISCUSSION

What are the Implications for the BWC?

Piers Millett, Biosecure Ltd.

Dr. Millett introduced the discussion session on potential implications of advances in biotechnology, synthetic biology, and biological design for the scope and operation of the BWC. As the workshop presentations illustrated, academic and industrial researchers from across the globe are developing microbial-based and bio-based production systems for biologics and chemicals. In some cases, these resources require significant investments (such as organism foundries) and may remain centralized in nature. In other cases, the development of cheaper benchtop-size and drop-in automated equipment may lead to wider distribution of capabilities. Which models, centralized versus distributed, will be followed by different types of technical advances over time remains an open question.

Advances in areas such as tool and platform development, automation, and experimental analysis are leading to progress on multiple fronts in design and development of biological production processes. However, the field is not yet at a stage in which a researcher could simply enter a desired end product into a software package, have the system map out the metabolic pathways, and robotically conduct the experiments necessary to achieve the desired result. A significant role remains for tacit knowledge and specialized resources. Practical challenges also remain in scale-up from laboratory to industrial-scale production of relevant microorganisms. Complex system aspects must be controlled, making it difficult for someone to switch from one route of production to another, whether that would entail use of a new organism, feeding an organism a new feedstock, or trying to produce a new end product. Each synthetic scheme would require intense optimization to achieve robustness and cost-effectiveness.

The discussion touched on the level of skill that would be required to take a typical laboratory organism and modify it to produce a chemical or biologic agent of concern. Speakers noted the

existence of simple kits for the expression of common genes such as green fluorescent protein in standard microbes such as *E. coli*, but indicated that modifying an organism into a pathogen remains difficult. Obtaining the biological parts, DNA sequences, plasmids, and other components is likely to present a major hurdle, as most would not be sold on the open market. The changing nature of biological production systems may also have implications for the types of evidence that might be used to identify an illicit BW program. It is hard to fully eliminate gene fragments, even with autoclaving. For the BWC, this means that there may be additional possible strategies to identify an attempted manufacture of a new pathogen.

Several positive implications of advances in bio-based production technology were also noted by participants. Technologies could be used to proactively mitigate risks associated with products that have off-target uses. For example, scientists could produce castor beans without ricin toxin to reduce the potential for production of ricin from the crop. Emerging biotechnologies can also be used to promote population defense against human and natural threats on a shorter timescale of days, rather than weeks or months. Significant time can be saved in vaccine production, for example, when a new pathogen can be sequenced and the data emailed to a vaccine production facility rather than shipping the pathogen itself around the globe.

Finally, participants noted the value of using data and concrete examples rather than abstractions when discussing potential biosecurity concerns, governance, and the BWC with academic and industrial communities. One strategy to promote ethical use of technologies is the establishment of norms within the scientific community for responsible conduct of science, safe laboratory practices that support biosafety/security, and awareness of prohibitions under the BWC and CWC. Self-governance mechanisms to counter potential risks can help demonstrate that scientists are willing to address safety and security concerns, with parallels being drawn to cybersecurity and community engagement in responsible practices. A number of suggestions were raised to foster engagement with industry, with one suggestion being as small as adding "We support the BWC" to company websites to bring the Convention into the conversation. Another participant noted that no company wants to be connected to potential BW risks and that companies would be wary of bringing attention to the topic. However, it was suggested that the issue could be framed as a part of corporate responsibility. The important role of champions in discussing biosecurity topics and of finding ways to encourage or incentivize companies to participate in such discussions was highlighted.

Continuing to engage academic and industrial communities in the BWC remains important and various models for achieving this goal could be considered. Several participants noted that bringing scientists and diplomats together is good in theory, but the scientific presentations can be complex and it is difficult to delve significantly into the implications for the BWC. Options to help address these challenges include use of working groups to consider issues presented by experts with the goal of presenting a digested version of the information to diplomats in a user-friendly and BWC-specific format. An additional strategy used by OPCW has been to host "Science for Diplomats" lectures on topics related to articles of the CWC, held over lunchtime between Convention sessions. Topics started at the level of "What is an atom? What is a molecule?" and now deal with S&T topics of more direct relevance to the CWC. Through the sessions, scientists and diplomats were able to build further trust and shared understanding. Opportunities for ongoing communication among academic and industrial scientists and policy-makers may help support nuanced discussions on the implications of scientific advances relevant to the work of the Biological Weapons Convention.

APPENDIX

AGENDA

10:30 **Welcome**
Katherine Bowman, U.S. National Academies of Sciences, Engineering, and Medicine

Introduction and Examples in Action
Detlef Männig, Evonik Industries
Alfred Szwarc, Brazilian Sugarcane Industry Association (UNICA)
Muhammad Farish Kamaludin, Malaysian Biotechnology Corporation

12:15 **Lunch**

13:45 **Reconvene**
Ryszard Slomski, Poznań University of Life Sciences

Strategies for Reducing Design and Development Barriers
Markus Herrgard, Technical University of Denmark
Sean Ward, Synthace Ltd.
Jason Kelly, Ginkgo BioWorks

What are the Implications for the BWC?
Piers Millett, Biosecure Ltd.
Discussion

16:30 **Adjourn**

DISCUSSION QUESTIONS

1. Why are diverse companies investing in advanced bio-based production capabilities for biological and chemical molecules – what is driving this trend forward and how it is changing the industry landscape (particularly for systems that are microorganism-based)?
2. How are new tools and systems making it easier to design and create microorganisms that produce specific biological or chemical molecules of interest?
3. What are the benefits of bio-based production technologies for the Biological Weapons Convention – for example, in responding faster or more flexibly to disease outbreaks or in developing therapeutics against emerging diseases?
4. Are any potential risks posed by these advances – for example, in making it easier to develop or produce a pathogen or toxin, or by increasing the numbers of people around the world skilled in implementing these techniques?
5. As universities and companies expand efforts to design microbial systems, how are the norms of responsible scientific conduct being communicated and promoted?
    a. Who should be responsible for communicating these norms?
    b. Are there mechanisms for assessing whether or not proposed research and development activities raise potential biosecurity concerns?
6. How can BWC policy makers keep abreast of developments in science and technology and learn about the views of academic and industry scientists?
    a. What system(s) do you think would work most effectively?
    b. For academic/industry scientists – what concerns would you want to convey to BWC policy makers (for example, in not unduly stifling innovation)?
    c. For policy makers – what concerns would you have for academic/industry participants (for example, in ensuring responsible development for beneficial purposes)?