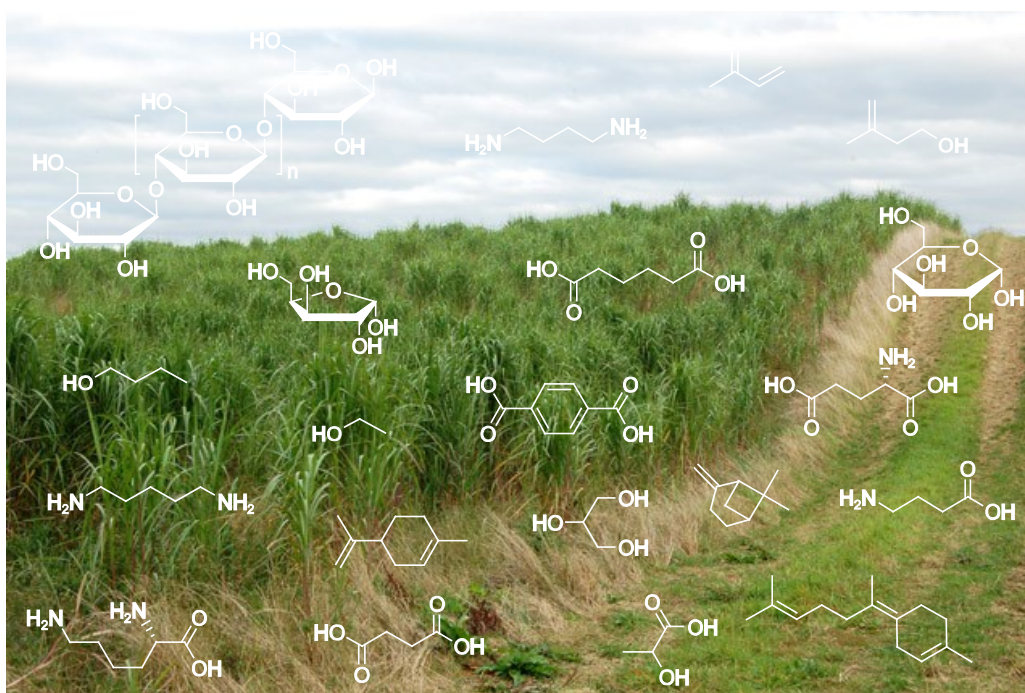


## Green Chemistry

*Mom, can green chemicals be produced by chemistry?*



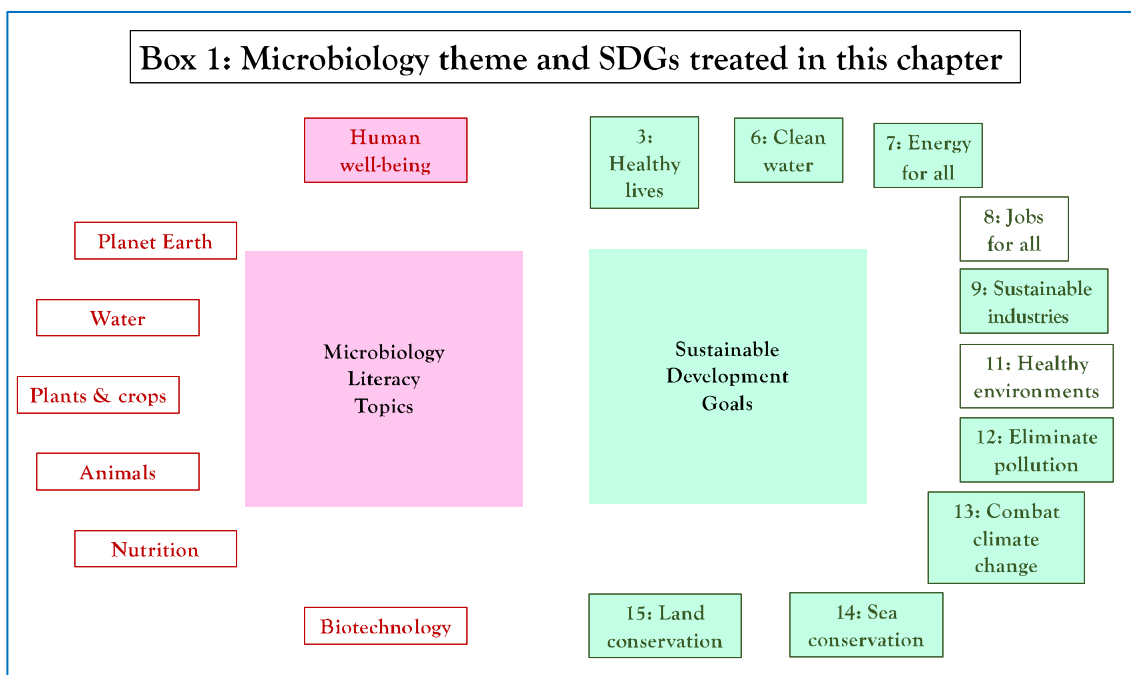
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## Green Chemistry

### Storyline

Chemicals are everywhere. From natural to synthetic ones, chemicals serve essential roles to maintain society of today through their use in various applications including food, medicines, fabrics, plastics, fuels, and raw materials for diverse industrial products. Most items around us today are products of the petrochemical industry, which uses fossil resources such as natural gas and crude oil as raw materials. However, our heavy dependence on fossil resources has significantly contributed to climate change and environmental pollution. Also, typical processes producing chemicals involve environmentally unfriendly and energy intensive conditions, often involving metal catalysts. In 1990s, the concept of **green chemistry** was introduced to address such issues and establish a sustainable chemical industry. Along with 12 principles of green chemistry, researchers have tried to design chemical products and processes that are safer to humans, animals, plants, and the environment. In this concept, microorganisms play important roles. Through the long evolutionary journey in diverse habitats and **niches**, microorganisms have acquired a myriad of **metabolisms** and **metabolites**. Researchers have explored such metabolic reactions and metabolites with the help of **metabolic engineering** to transform microorganisms into living factories producing diverse compounds from renewable feedstock. In the foreseeable future, the traditional **petroleum refinery** is expected to be replaced with the environmentally-friendly **biorefinery**, successfully following the 12 principles of green chemistry. Also, biorefineries will help us to achieve **Sustainable Development Goals**.



### The Microbiology and Societal Context

*The microbiology:* microbial diversity and metabolic diversity; microbial cell factory; metabolic engineering; microbial fermentation; biorefinery; renewable feedstock; mild process conditions; whole cell catalyst; biocompatible/biodegradable products; biosecurity. *Sustainability issues:* health and well-being; clean water; clean energy; industry and infrastructure; sustainable cities

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and communities; responsible consumption and production; climate action; life in water; life on land.

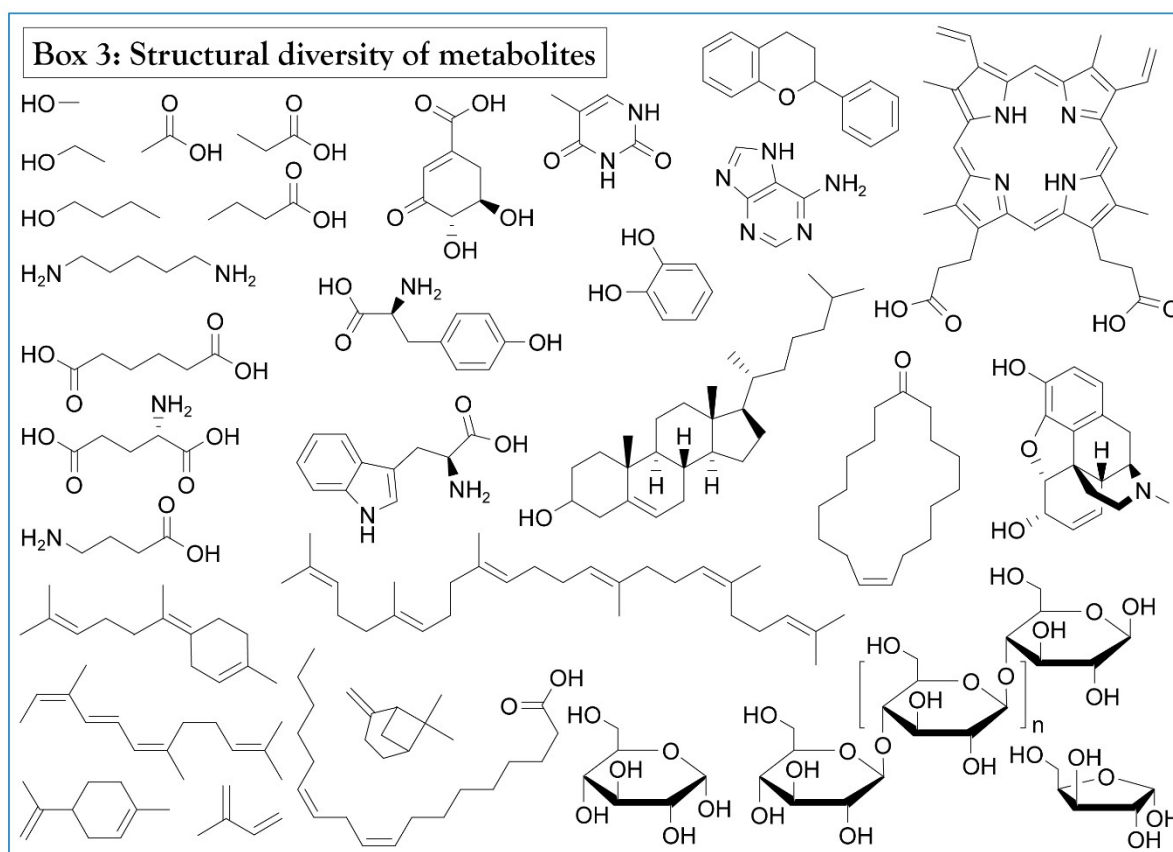
### Box 2: Twelve Principles of Green Chemistry

1. **Prevent waste:** avoid waste production rather than treating or cleaning up after generation.
2. **Maximize atom economy:** design synthetic methods to maximize incorporation of all materials used in the process into the final product.
3. **Design less hazardous chemical syntheses:** develop synthetic strategies to use and generate substances with little or no toxicity to human health and the environment.
4. **Design safer chemicals:** obtain chemical products retaining full functionality while reducing toxicity.
5. **Use safer solvents and auxiliary agents:** avoid using aggressive solvents, separation agents, and other auxiliary agents; use innocuous agents whenever possible.
6. **Design for energy efficiency:** novel methods to minimize energy requirements; run synthetic reactions at ambient temperature and pressure rather than extreme conditions.
7. **Use renewable feedstock:** use renewable raw materials or starting materials (e.g. agricultural products, wastes of other processes) rather than depleting materials (e.g. petroleum products, mining products).
8. **Reduce chemical derivatives:** avoid using blocking groups, protection/deprotection, or temporary modifications as such steps require additional reagents and often generate waste.
9. **Use catalysts rather than stoichiometric reagents:** minimize generation of waste by employing catalysts, which facilitate a reaction multiple times, rather than employing stoichiometric reagents that mediate a reaction only once at excess concentration.
10. **Design products to degrade after use:** design chemical products to break down to innocuous compounds after use that do not persist in the environment.
11. **Analyze in real-time to prevent pollution:** develop real-time, in-process monitoring and control during syntheses to prevent or minimize formation of hazardous substances.
12. **Design processes to prevent accidents:** choose substances and their phases (e.g. solid, liquid, or gas) to minimize the potential for chemical accidents, including explosions, fires, and release to the environment.

### Green Chemistry: the Microbiology

1. *Microorganisms produce structurally diverse chemicals with stereospecificity.* Traditional chemical industries have heavily depended on petroleum refining and struggled to synthesize valuable chemicals with complex chemical structures. However, the majority of chemicals obtained from petroleum refineries have relatively simple chemical structures with

limited diversity. To produce a complicated molecule from such petroleum-derived building block chemicals, organic chemists used to devise a series of chemical reactions, where most steps include toxic components. In contrast, microorganisms possess structurally diverse metabolites, ranging from small organic acids to complicated secondary metabolites. Some metabolites themselves are already valuable compounds, while other metabolites serve as scaffolds for syntheses of useful molecules. On the other hand, many useful compounds have sibling chemicals called **stereoisomers**, in which their atoms are connected identically, but their three-dimensional arrangements are different. Although the chemical structures of stereoisomers look similar to each other, they often behave differently in biological systems, for example, one stereoisomer may act as a powerful medicament, whereas another may lack activity, so only one stereoisomer may be preferred for an application. Nevertheless, many chemical products are generated as a mixture of stereoisomers, since synthesis and purification of **stereospecific** products are usually complicated and costly. In contrast, microorganisms are good at producing and distinguishing stereoisomers and frequently prefer producing a single bio-active stereoisomer. Collectively, structural diversity and stereospecificity of microbial metabolites enable easier production of complicated and valuable compounds.



2. **Microbial cells can turn into living factories producing diverse chemicals.** A myriad of microbial species have adapted to diverse habitats and **niches** on the earth. During evolution, microorganisms have expanded their metabolisms and acquired an uncountable number of metabolites. Biorefineries exploit such capabilities of microorganisms to produce valuable chemicals and replace traditional chemical industry rooted in the petrochemical refinery. Some microorganisms that naturally produce certain useful chemicals in large quantity (i.e. **overproduction**) have been employed in commercial fermentative processes (note that in

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biotechnology, **fermentation** often refers to cultivation of microorganisms to produce target product in either anaerobic or aerobic condition). For example, brewer's yeasts have been employed to produce bioethanol, and an anaerobic bacterium *Clostridium acetobutylicum* has been employed to produce mixture of acetone, butanol, and ethanol since the World War I, through a characteristic fermentative process called acetone-butanol-ethanol (ABE) fermentation. Through the development of metabolic engineering in the 1990s, the production capacities of the natural overproducers could be enhanced by reorganizing metabolism through genetic manipulation of the host organisms. Moreover, microorganisms incapable of overproduction can be transformed into overproducers of target products of commercial interest. Engineering to produce **unnatural chemicals** (i.e. chemicals heterologous to the host microorganism) and even **non-natural chemicals** (i.e. chemicals not found in the nature) is possible as well. Such microbial cell factories can produce wide range of chemicals, including **building block chemicals** for chemical industries, plastics, fuels, nutraceuticals, food additives, cosmetics, and medicines.

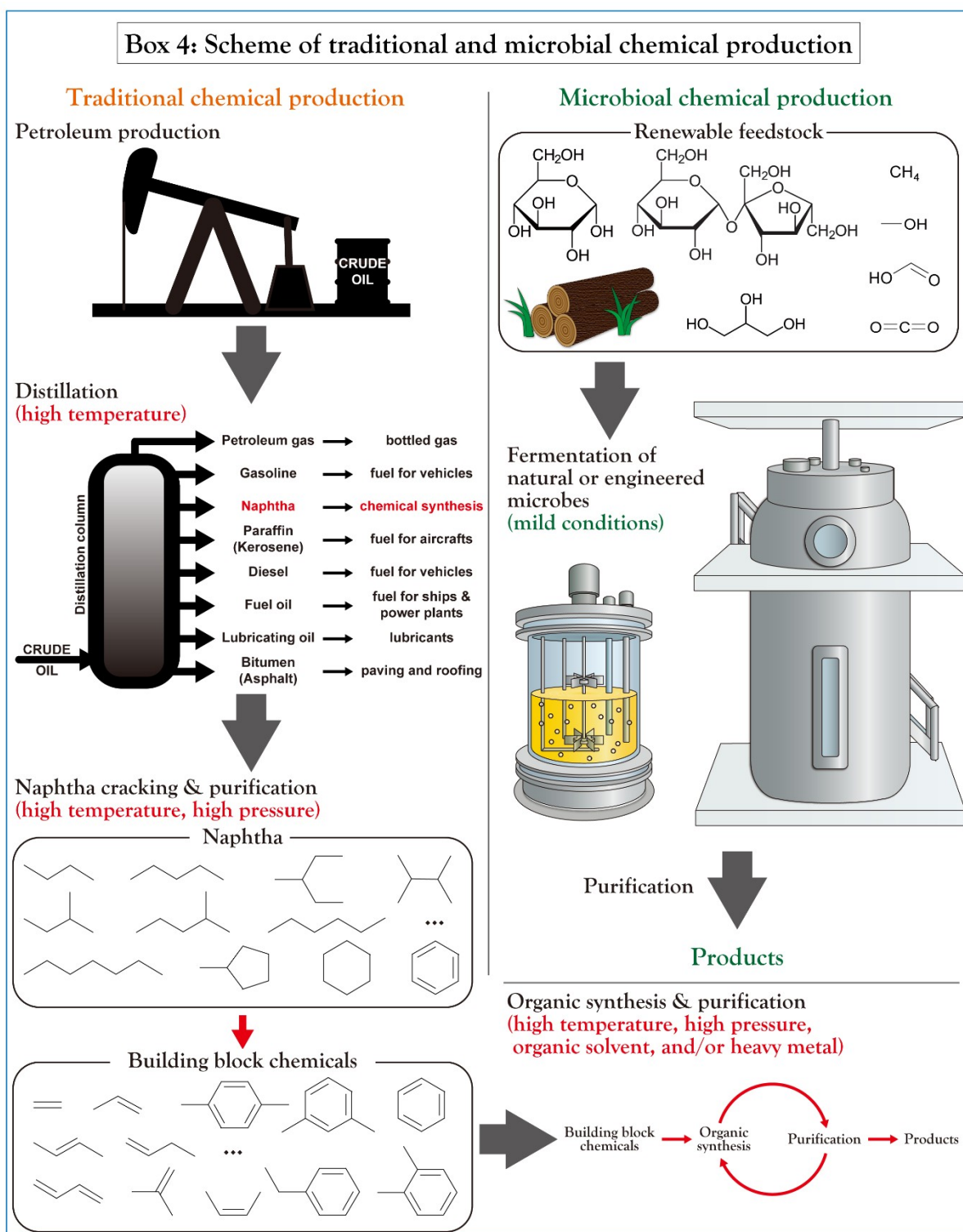
3. ***Microbial cell factories utilize renewable feedstocks to produce value-added compounds.*** Most traditional chemical industries use chemical compounds derived from petroleum as raw materials, which is a non-renewable carbon reservoir in the periphery of the **global carbon cycle**. Thus, traditional chemical industries are not sustainable and exacerbate climate change by releasing carbon from fossil fuel reservoirs to the atmosphere. Biorefineries based on microorganisms, however, utilize **renewable carbon sources**, including sugars derived from non-edible biomass (e.g. lignocellulosic biomass), lipids and fatty acids derived from food waste, and glycerol generated as byproduct of biodiesel factories. In addition, carbon dioxide, a major **greenhouse gas**, can be directly assimilated to produce value-added compounds. Thus, microbial cell factories are contributing to sustainable chemistry (see principle 7 of green chemistry).

4. ***Microbial fermentation to produce chemicals is conducted under mild, innocuous conditions without addition of hazardous agents.*** Many chemical reactions employed in traditional industries require extreme conditions, such as high temperature, high pressure, and high/low **pH**. In addition, reactive/toxic agents are used for efficient conversion of precursors to product chemicals. Organic solvents are often used as well for reaction efficiency. Furthermore, catalysts used to facilitate chemical reactions and allow milder reaction conditions are mostly composed of **heavy metals**, which are toxic to humans, animals, plants, and the environment. In contrast, microbial fermentative processes are conducted at ambient pressure, room to warm temperature (30-37°C), and neutral to slightly acidic/basic pH, contributing to energy efficiency and safety of chemical processes (see principles 3, 6, and 12 of green chemistry). In addition, all chemical reactions – except those that occur spontaneously – are catalyzed by **enzymes**, not requiring use of toxic agents and organic solvents of traditional chemistry (see principles 3, 5, 8, 9, and 12 of green chemistry). The absence of toxic agents and solvents during the fermentative processes obviates the generation of hazardous wastes (see principles 1 and 11 of green chemistry). This is particularly beneficial in the production of nutraceuticals, food additives, cosmetics, and medicines that humans directly consume, since contamination with toxic compounds is absent from the manufacturing process.

5. ***Microorganisms can be transformed into whole-cell catalysts that convert precursors into products at high yields.*** Many chemical processes have been commercialized with high conversion yields. Alternatively, microbial cells can be engineered into whole-cell catalysts to

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achieve **conversion yields** comparable to or exceeding those of conventional chemical processes (see principle 2 of green chemistry). One good example is conversion of *p*-xylene into terephthalic acid, which is an important monomer for the production of polyethylene terephthalate (PET) used to make PET bottles. Currently, most terephthalate is produced by oxidizing *p*-xylene through the Amoco process which employs glacial acetic acid, catalysts composed of manganese and cobalt salts, and bromide compounds. All of these chemicals are either toxic or difficult to degrade and recycle. A typical conversion yield of *p*-xylene to terephthalate in the Amoco process is above 95 **mol%**. On the other hand, an engineered *Escherichia coli* strain achieved a conversion yield of 96.7 mol%, in a two-phase fermentative process consisting of an aqueous phase and an organic phase consisting of biocompatible oleyl alcohol.



6. *Microbial cell factories can conduct a sequence of multiple chemical reactions catalyzed by enzymes in a single-step fermentation process.* In chemical industries, chemicals are often synthesized through a series of conversion reactions. Frequently, each reaction step requires different conditions, agents, and catalysts. Because such agents and catalysts may be rather unspecific and interact with a broad range of substrates, multiple separation/purification steps and derivatization steps are necessary, to prevent undesired interactions with the intermediates obtained at different stages. The need to separate these



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different stages complicate chemical manufacturing processes and generate additional wastes as the cost of high yield and efficiency. In contrast, enzymes have high specificities toward substrates, and do not generally affect non-target intermediates, so multiple conversion steps catalyzed by enzymes can be simultaneously conducted inside a single cell during microbial production of chemicals. In addition, feeding a renewable feedstock is enough to grow microbial cells, biosynthesize all infrastructure of the microbial cell factories (i.e. enzymes and coenzymes of the enzymes), and supply metabolites for chemical product formation. These aspects of microbial chemical production contribute to reducing generation of wastes during chemical syntheses (see principles 1, 8, and 9 of green chemistry).

7. ***Microbial products enable replacement of traditional chemicals and materials with safe, biocompatible, and biodegradable substitutes.*** Many chemical products with outstanding properties have been commercialized through chemical industries. However, a significant number of such products have endangered health of humans, animals, plants, and the environment. For example, plastics have greatly contributed to manufacturing and are used everywhere in modern societies, yet non-degradable plastic wastes and **micro/nanoplastics** are polluting lands/waters and threatening lives on the earth. To overcome such issues, microbial polymers called **polyhydroxyalkanoates (PHAs)** and their biosynthetic pathways/machineries have been exploited to produce biodegradable plastics with desired properties, such as biocompatibility, durability, and flexibility. In addition, microbial cellulose was found to be a good substitute of current polyolefin-based plastic membrane used as separator in lithium batteries. Moreover, microorganisms have been engineered to produce spider silk – a natural fiber much stronger than the same weight of steel. The resulting spider silk can replace nylon and Kevlar that are used to fabricate bulletproof vests and parachutes. Such **bioproducts** and their microbial overproducers are contributing to solve the health and environmental pollution issues that our generation is facing without compromising the quality of life (see principles 4 and 10 of green chemistry).

8. ***Biocontainment technologies prevent release of genetically engineered microorganisms to the environment.*** Many microbial overproducers are genetically engineered, and concern has been expressed that their accidental release to the environment may result in unexpected outcomes. In addition, high-performance strains should be secured from competing companies, since the overproducer strains are key intellectual properties of biorefinery companies. To address such biosecurity issues, **biocontainment** technologies have been actively studied to allow survival and operation of the microbial cell factories only in the specific commercial environmental conditions designed by the company.

### Relevance for Sustainable Development Goals and Grand Challenges

The microbial dimension of green chemistry relates to several SDGs (*microbial aspects in italics*), including

- **Goal 3. Ensure healthy lives and promote well-being for all at all ages** (*improve health, reduce preventable disease and premature deaths*). The absence of hazardous agents used



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during microbial production of chemicals guarantees safe working conditions in the manufacturing environments. In addition, absence of toxic waste generated during the chemical synthesis prevents pollution of the atmosphere and the environment, contributing to the health and well-being of inhabitants nearby the factories. Biocompatible and biodegradable materials produced by microbial cell factories also prevent pollution of the environment after their use and eventually benefit the health of people.

- **Goal 6. Ensure availability and sustainable management of water and sanitation for all** (*assure safe drinking water, improve water quality, reduce pollution, protect water-related ecosystems, improve water and sanitation management*). Design and production of safer and biocompatible compounds, such as biodegradable plastics, can prevent release of toxic compounds and non-degradable wastes into surface waters, and thereby protect them from contamination, and resulting contamination of groundwater supplies into which they percolate.
- **Goal 7. Ensure access to affordable, reliable, sustainable and modern energy for all** (*ensure access to clean, renewable and sustainable energy, and increase energy use efficiency*). Mild process conditions during biorefinery save energy used to operate chemical plants. In addition, the production and consumption of biodegradable materials can save energy needed to remove or clean up non-degradable materials disposed.
- **Goal 9. Build resilient infrastructure, promote inclusive and sustainable industrialization, and foster innovation** (*develop sustainable industry*). Biorefineries employ microbial cell factories to utilize renewable feedstock for chemicals production, thereby reducing the consumption of fossil fuels and the release of the carbon locked up in them (i.e. the oil field) to the environment. In addition, the independence of biorefineries from toxic chemicals and solvents avoids environmental pollution, and contributes to the sustainability of chemical industries.
- **Goal 12. Ensure sustainable consumption and production patterns** (*achieve sustainable production and use/consumption practices, reduce waste production/pollutant release into the environment, attain zero waste lifecycles, inform people about sustainable development practices*). Microbial production system helps establishing sustainable industries by utilizing renewable carbon sources as raw materials to produce safer and eco-friendly chemicals/materials through greener processes. In addition, they provide consumers better choices for sustainable consumption.
- **Goal 13. Take urgent action to combat climate change and its impacts by regulating emissions and promoting developments in renewable energy** (*reduce greenhouse gas emissions, mitigate consequences of global warming, develop early warning systems for global warming consequences, improve education about greenhouse gas production and global warming*). Chemical production by fermentative processes takes place under mild conditions (i.e. ambient pressure and room to warm temperature), saving the energy required to reach and sustain the high pressures and temperatures characteristic of classical chemical processes. Use of less energy reduces the amount of fuels combusted to run chemical factories and prevents release of greenhouse gas to the atmosphere. Green chemistry has a lower carbon footprint and hence contributes to efforts to slow down climate change.

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- **Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development** (*reduce pollution of marine systems by toxic chemicals/agricultural nutrients/wastes like plastics, develop mitigation measures for acidification, enhance sustainable use of oceans and their resources*). The eco-friendly processes and safer products of biorefinery reduces the release of hazardous chemicals to the environment and eventually to the ocean. In addition, the design and production of biodegradable plastics prevents accumulation of plastic wastes and spread of microplastics to the ocean. Moreover, the release of endocrine disrupting – hormone-like – chemicals and their accumulation in marine organisms is avoided. These features help conserve marine environments, resources, and organisms.

### Potential Implications for Decisions

#### 1. *Individual*

- a. Consume cheaper products of traditional chemical industries or greener products of bioindustries (**bioproducts** have relatively higher price while being better for health and the environment than traditional chemical products)?
- b. Pursue convenience or favor the environment (single-use products are convenient yet generate environmental costs during the production and after use)?

#### 2. *Industrial policies*

- a. Continue traditional production systems or adopt biorefineries (**bioproduction** of chemicals are more sustainable but needs investment and increases production costs)?
- b. Use edible biomass or non-edible biomass as raw material (sugars derived from edible biomass are more efficient for chemical production yet aggravates food shortage issues)?
- c. Best location of factories regarding logistics (source of renewable feedstock and location of major markets should be near the factories, as transportation of raw materials and products increases **carbon footprints** of the products and contributes to the climate change and environmental pollution)

#### 3. *National policies relating to green chemistry*

- a. Health/environmental advantages and disadvantages upon transition of current chemical industries toward biorefineries
- b. Greenhouse gas production and climate change
- c. Ensuring safe drinking water supplies (pollution of underground water due to toxic chemicals released from non-degraded products buried underground)
- d. Environmental pollution (pollution of air, lands, rivers, and oceans)
- e. Conservation of marine/terrestrial animals
- f. Develop evidence-based policies for genetically modified organisms and their products

### Pupil Participation

#### 1. *Class discussion of the issue associated with green chemistry*

- a. Which products around you are produced by microbes?
- b. Are chemicals and materials produced by genetically modified microorganisms safe?
- c. How are the wastes that we discard everyday cleaned up?

#### 2. *Pupil stakeholder awareness*

- a. Production and use of single-use food containers are environmentally costly. On the other hand, production of multi-use food containers and washing to reuse also have environmental costs. Which is more harmful for the environment?
- b. Clothes and toys are products of chemical industries, and their production contributes to environmental pollution and the climate change. Which actions can you take for sustainable consumption?
- c. What are the benefits of non-degradable products and why are they causing problems in the environment?

#### 3. *Exercises*

- a. Plastic wastes are threatening marine organisms. What can you do to help them?
- b. You entered a shop to buy a pen, and you found two products. One is made of conventional plastic and the other one is made of biodegradable plastic but has higher price. Which one would you buy?
- c. What can we contribute to making chemical industries more sustainable?
- d. Which chemicals and materials are commercially produced using microbial cell factories?

### The Evidence Base, Further Reading and Teaching Aids

#### 1. Green chemistry

<https://www.epa.gov/greenchemistry/basics-green-chemistry>;

<https://www.acs.org/content/acs/en/greenchemistry.html>;

#### 2. Biorefinery

<https://www.bioenergyconsult.com/biorefinery/>;

#### 3. Metabolic engineering

[https://en.wikipedia.org/wiki/Metabolic\\_engineering](https://en.wikipedia.org/wiki/Metabolic_engineering);

### Glossary

**absorbance:** degree of absorption of light of specific wavelength.

**agent:** chemical involved in a chemical reaction.

**auxiliary agent:** auxiliary chemical or substance that is used to support process to synthesize or obtain desired chemical product.

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**biocompatible:** biocompatible materials do not provoke immune responses, and hence rejection, when introduced inside a living system.

**biocontainment:** technologies used to restrict a specific organism to a fixed space, in order to prevent its survival outside of this space, or undesired use by third parties.

**biodegradable:** material that can be degraded by living organisms.

**bioproduct:** compound or material produced by biological systems.

**bioproduction:** production of desired compounds and materials using biological systems.

**biorefinery:** a discipline of producing useful chemicals and materials from renewable feedstock with the help of biological systems.

**biosecurity:** all aspects of security related with biological systems, such as issues related to pathogens, antibiotic-resistant bacteria, and microbial strains used in industries.

**biosynthesis:** biological processes generating chemicals inside or by a living cell.

**blocking group:** a moiety in a chemical compound that protects reactive part of the compound from other reactive agents.

**building block chemical:** chemical used as precursor for synthesizing various important chemicals in chemical industries.

**carbon footprint:** total amount of carbon dioxide generated during the production and transportation of a product.

**catalyst:** compound or material used to facilitate a chemical reaction.

**conversion yield:** ratio of precursors converted to products in a chemical reaction.

**derivative:** chemical modified from an original chemical.

**deprotection:** removing a moiety of compound used to protect a reactive part of the compound to restore the reactive part.

**enzyme:** biological compound facilitating a specific chemical reaction.

**fermentation:** biological process occurring as microorganisms grow under anaerobic conditions (i.e. absence of oxygen); in biotechnology, any cultivation of microorganisms for production of desired compounds is called fermentation regardless of the presence/absence of oxygen.

**global carbon cycle:** global flow of carbon in various forms (e.g. carbon dioxide, organic materials, inorganic carbonate salts)

**green chemistry:** trend in chemistry to make chemical reactions, process, and industries sustainable.

**greenhouse gas:** gas compound, such as carbon dioxide and methane, contributing to the greenhouse effect and accelerating climate change

**heavy metal:** a group of metals with high density; heavy metals are inefficiently excreted from body and their accumulation in body causes various symptoms and diseases.

**intermediate:** chemical species other than reactants and products that temporarily appears and is consumed in chemical reactions.

**metabolic engineering:** a discipline engineering metabolism of biological system to achieve a desired goal, such as overproduction of valuable chemicals.

**metabolism:** the spectrum of chemical reactions that take place inside living organisms.

**metabolite:** a chemical that appears inside living systems.

**microplastic:** micrometer ( $10^{-6}$  meter)-sized particles of plastic formed from plastic released into the environment

**mole:** a measure of counting the quantity of atoms and compounds; 1 mole (=1 mol) is equivalent to  $6.02 \times 10^{23}$  molecules.

**mol%:** a measure to quantify percentage (%) of chemical species in a heterogeneous chemical mixture based on their frequency in moles.

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**niche:** an environment with physical, chemical, and biological conditions distinguished from surroundings.

**nanoplastic:** nanometer ( $10^9$  meter)-sized particles of plastic formed from plastic released into the environment

**non-natural chemical:** chemicals not found in the nature.

**nutraceutical:** compounds that have health benefits; nutrition + pharmaceutical.

**overproducer:** an altered organism that produces more of a specific compound than usual.

**petroleum refinery:** a process of producing various chemicals and materials using petroleum as the starting material.

**pH:** measure of acidity or alkalinity; solution with pH equal to 7 (i.e. pH 7) is neutral, solution with pH less than 7 is acidic, and solution with pH higher than 7 is alkaline; more deviation pH from 7 means stronger acidity or alkalinity.

**phase:** a region of matter consisting of similar physical/chemical properties; a form of material, such as solid, liquid, and gas.

**polyhydroxyalkanoate (PHA):** biological polymer that is generated by microorganisms to store excess carbon source for future use as energy source.

**protection:** adding a chemical structure (i.e. blocking group) to prevent a reactive part of a compound from reacting with other reactive agents.

**renewable carbon source:** organic chemical species that can be easily biosynthesized in nature from carbon dioxide.

**renewable feedstock:** starting materials of chemicals industries, which can be repeatedly obtained from nature.

**stereoisomers:** a group of chemicals of which constituting atoms are connected in the same manner but arranged differently in three-dimensional space

**stereospecific:** a stereospecific product consists of only one type of stereoisomer

**solvent:** liquids used to dissolve solid compounds; organic liquids used to dissolve solid compounds and conduct reactions.

**stoichiometric:** a stoichiometric reaction of chemical species occurs at a fixed molar ratio, e.g. one-to-one, in contrast to catalytic, in which the catalyst species is repeatedly re-used in the chemical conversion.

**substrate:** a chemical species that is bound by an enzyme and converted to another chemical species (i.e. product), through an enzymatic reaction.

**Sustainable Development Goals:** 17 goals announced by United Nations to achieve sustainable development of the world.

**unnatural chemical:** chemicals not found in a certain organism.