

Developing Neo-bioplastics for the Realization of Carbon Sustainable Society

Akihito Nakanishi^{1,2#*}, Kohei Iritani^{3#}, Yuri Sakihama⁴

¹Graduate School of Bionics, Tokyo University of Technology, Hachioji, Japan

²School of Bioscience and Biotechnology, Tokyo University of Technology, Hachioji, Japan

³Department of Applied Chemistry, School of Engineering, Tokyo University of Technology, Hachioji, Japan

⁴Tokyo University of Technology, Hachioji, Japan

#Co-first author

*Correspondence should be addressed to Akihito Nakanishi; nakanishiah@stf.teu.ac.jp

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Abstract

With the global economic development, the use of plastic has increased significantly. Currently, over 8.3 Gt of plastic is produced annually and approximately 6.3 Gt is discarded. Furthermore, 99% of the plastic is obtained from petroleum. When the plastic is obtained from petroleum, the carbon moves in one direction from the underground into the atmosphere in the form of CO₂ gas, which is unsustainable, thereby requiring the development of a carbon recycling process that is independent of oil. The plastic waste is inadequately treated, which results in a substantial amount of plastics being released into the environment, thereby creating micro- and nano-plastic problems. Although bioplastics, such as polylactic acids, polyhydroxyalkanoates, and polybutylene succinate, are being actively researched and developed as alternatives to petroleum-based plastics, they have not been able to replace petroplastics yet due to their complex production processes and high cost. Therefore, we develop an alternative method for manufacturing bioplastics, wherein cells of unicellular green algae are used as the raw material; the plastics obtained are known as cell-plastics. The direct use of green algal cells for plastic production has several advantages. Through photosynthesis, green algae use CO₂ as the carbon source to construct new cells; therefore, depending on the rate of CO₂ assimilation, the growth activity of the grown cell is greater than that of the ordinary terrestrial plants. Algal cells have shown potential for application in robust cell-plastics owing to their rigid cell wall structure. Although cell-plastics are currently under trial in laboratory, we believe that they will be suitable for industrial-scale production.

Keywords: Sustainable society; Carbon recycle; Bioplastics; Unicellular green alga; Cell-plastics

Plastic Production

Plastic is a composite material made from organic polymers and can be freely molded into a film, fiber, or plate by heat or pressure processing. In 1839, Goodyear proposed the vulcanization of natural rubbers, while E. Simon discovered polystyrene [1]. From the viewpoint of mass production and cost-effectiveness, low-density polyethylene (LDPE) [2-4], high-density polyethylene (HDPE) [4-6], polypropylene (PP) [7-10], polyvinyl chloride (PVC) [11-13], polystyrene (PS) [14-16], and polyethylene terephthalate (PET) [17-19] have been recognized as general plastic materials. In addition, polymers that show heat-resistant temperature over

100°C and high tensile strength over 40 MPa have been categorized as engineering plastics, such as polyamide [20,21], polyacetal [22,23], and polycarbonate [24,25]. Overall, the use of resins that are synthesized by chemical methods have increased considerably since 20th century. In the 1940s, manufacturing industries developed owing to the improvement in the basic properties of polymers such as mechanical strength, flexibility, plasticity, heat- or chemical-registration. However, owing to the recent improvement in the quality of life, the mechanical strength of plastics has become crucial because plastics can replace metal or ceramic materials owing to their low cost. Plastics are used in several fields, including (1) the daily necessities such as bottles, packing supplies, grocery bags, clothes,

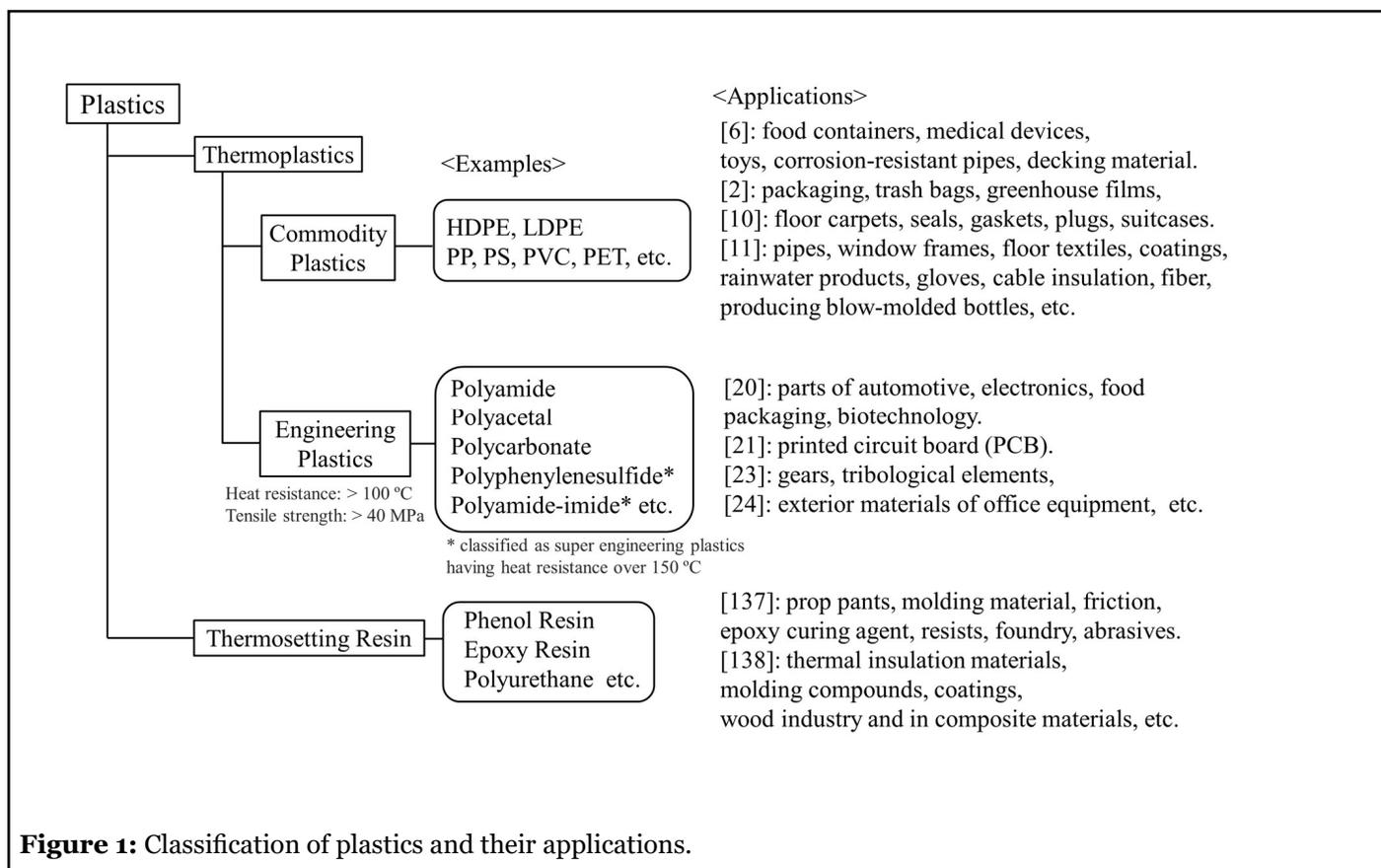


Figure 1: Classification of plastics and their applications.

furniture; (2) electronic devices, automobiles, and home appliances; and (3) aerospace materials (Figure 1). Plastic materials have become an indispensable part of the modern society, with 99% of the plastic materials being produced from petroleum, which is a non-recycled carbon resource [26]. In 2015, the cumulative plastic production worldwide was reported to exceed 8.3 Gt [27], which suggests that the amount of oil used was approximately the same as that of the plastics produced.

Plastic Disposal

In recent years, the total amount of global plastic waste reached 6.3 Gt/year, which occupies an approximate volume of 5.9 km³ [27]. The increasing global population requires further production of plastics, thereby resulting in a significant increase in the amount of plastic waste. The amount of plastic waste is expected to reach up to 12 Gt by 2050 [27]. The disposal process of the plastic wastes is such that only 21% of the total plastic can be recycled or incinerated, and therefore, the rest is left untreated the environment [27]. When plastic waste is incinerated, CO₂ gas, which contributes to the greenhouse effect, toxic chemical-ashes, and slag, is released into the environment, thereby damaging the environment due to the greenhouse effect and the effects of other pollutants

[28]. Plastic incineration processes emit 400 Mt of CO₂/year into the atmosphere, which seriously impacts the environment. In contrast, since most untreated plastic waste is controlled as normal waste by the authorities, systematic control of plastic waste for storage and degradation is difficult, even with progressive controls in urban areas. Due to waste-disposal mismanagement, significant amounts of plastic waste have been released and accumulated in the environment. Furthermore, 4% of the waste plastic is transported into rivers by rainwater, which eventually reaches the sea. Therefore, there is a considerable contamination load stress on the biological ocean-environment [29-32]. In the ocean, large amounts of degraded plastic patches have accumulated, which have resulted in death of the ocean organisms and food insecurity [33]. The plastic waste remains in the environment for a long time owing to their high durability. For instance, several research have reported that the plastic materials (e.g., bottles, containers, and trays) made of PET via petrochemistry have maintained their form in the environment for 90 years [34,35]. Another research has reported that the effects of these plastic wastes on the environment will worsen increasingly in the future [36]. Therefore, plastic contamination for a long duration affects biogeocenosis and causes serious problems, such as environmental pollution and destruction of ecosystem [37].

Risk of Micro Plastics

Along with the accumulation of plastic wastes on land, the environmental pollution caused by microplastics in the ocean is also concerning. Microplastics are defined as plastic pieces with diameter of 5 mm or less [38]. Previous reports have shown that approximately 13% of plastic refuse is classified as microplastics [38]. Among them, the plastic pieces with diameters of 1.01–4.75 mm have an adverse effect on the marine ecosystem, especially the food chain. Although the area occupied by microplastics in the Pacific Ocean is approximately 1% (1.6 million km²/165 million km²), the problem of environmental pollution by microplastics is of global significance because the affected area is spreading rapidly [39]. According to another study, the microplastics spread in the oceans have a density of 1×10^6 pieces/km² [38]. Microplastics have been found in temperate oceans and near the North and South Pole [38]. Furthermore, the microplastics have been found on the sea floor and in sediment [40-42].

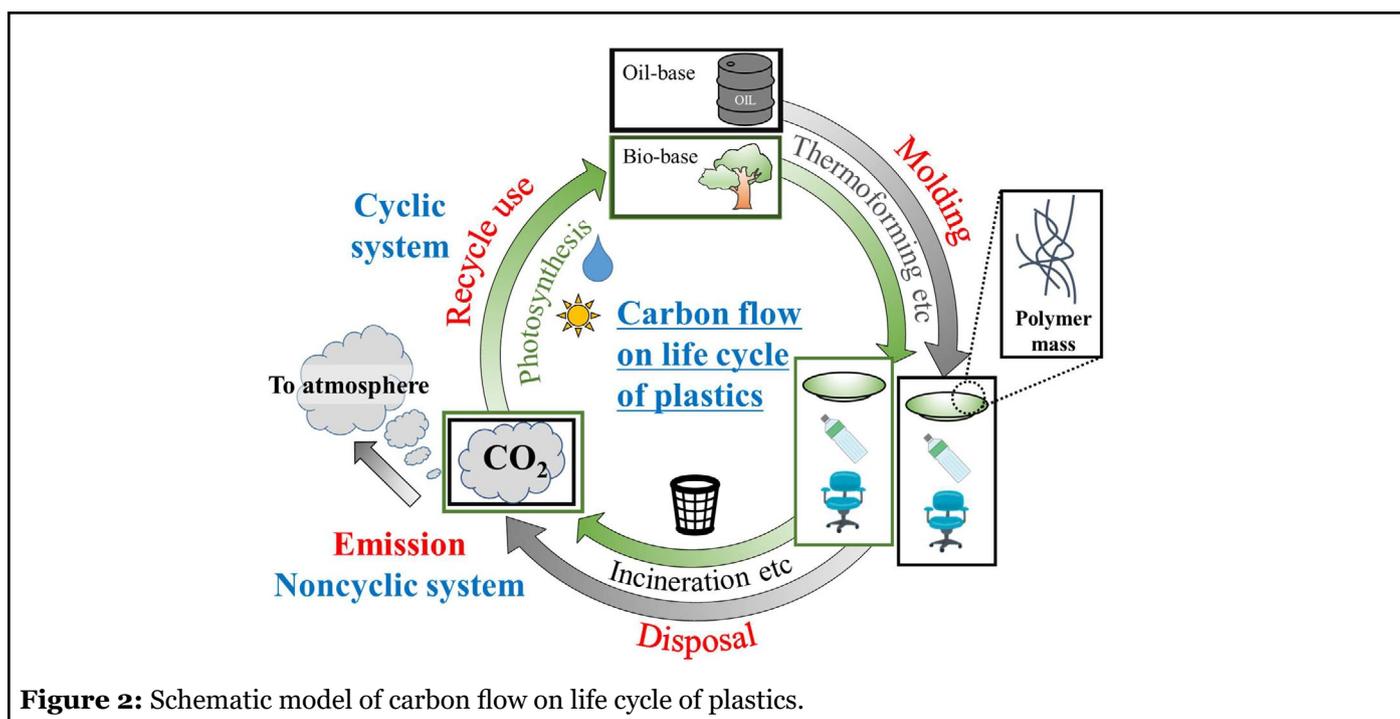
Recently, the environmental load of general plastics, such as PE, PP, PS, and PET [43-45], was investigated from the viewpoint of environmental science, microbiology, genetics, and toxicology [46-49]. In addition, it was revealed that organisms in the oceans, which ingested microplastics, experienced induced disruption of biological processes, gastrointestinal irritation, changes in the microbiome, lipid metabolism, and oxidative stress [50-52]. Moreover, besides damage and inflammation to living things themselves, they contain several kinds of environmental pollutants, such as endogenous plastic additives,

metals, polycyclic aromatic hydrocarbons, chlorinated hydrocarbons, and pathogenic microorganisms. From the viewpoint of human food, microplastics have been found in fish, salt, beer, sugar, and bottled water [49]. Consumption of food that contain microplastics increases the risk of cancer and other diseases [53]. In addition, the mobility of harmful substances in the human body is accelerated by inhalation of microplastics, which increases the possibility of serious illness due to biological concentration.

Requests for Bioplastics as Sustainable Sources

Owing to the serious global problems related to the plastic waste, China, which is one of the countries that accepted plastic wastes, declared that it will no longer be receiving waste from other countries for recycling after December 31, 2017 [54]. Global actions, such as the Chinese declaration, require the development of plastic materials using non-petroleum resources. Therefore, bioplastics have been chosen as alternatives to oil-based plastics. In the near future, approximately 11 Mt of the oil-based plastics are expected to be replaced by the bio-based variant [54].

With regard to the expansion of a circular bioeconomy, research and development of recyclable plastics that can degrade into CO₂ without any byproducts have attracted international attention (Figure 2). From the viewpoint of circular bioeconomy, the balance between resources and plastic waste can be maintained by recycling plastic through appropriate treatment methods. Although there has recently been some improvement in the recycling



of plastic waste, the success has been minor despite an increase in global intentions for plastic recycling. Note that the ratio of plastic recycling globally is approximately 20% [27], whereas in Europe, it is only 6% [55]. Therefore, research and development based on the direct conversion of plastic waste to useful plastic materials and carbon source conversion via CO₂ in biogeocenosis has attracted significant attention worldwide. For instance, Europe, which has a strong desire for plastic reduction, realizes 25.8 Mt of plastic waste per year, with 59% of the plastic waste derived from packaging materials [28]. Therefore, the replacement of packaging plastics with a circular resource will resolve a major part of their environmental problems. Following the damage to the environment that has resulted from the production and discarding of plastics, extensive environmental campaigns have been conducted for minimizing plastic waste. Therefore, over the last 20 years, academic communities have made progress in the development of bioplastics as circular resources [56,57]. Although the main discussions in the research have been whether plastics maintain their physical and chemical properties for commercial use and if biodegradability violates the criterion for industrial use [57,58]. Thus far, no developed bioplastics have met all the criteria [58,59]. Therefore, plastic production (including bioplastics) and the associated environmental effects, along with acute effects on developments and dwellings, should be reconsidered to curb plastic pollution and realize sustainability [60-62].

Definition of Bioplastics

Based on the raw materials and biodegradability, plastics are classified as follows: (a) Oil-based with low biodegradability; (b) oil-based with high biodegradability; (c) biomass-based with low biodegradability; and (d) biomass-based with high biodegradability (Figure 3). Bioplastics are defined as either highly biodegradable plastics, i.e., (b) and (d), or plastics of categories (b), (c), and (d). In this study, we treat the latter definition as bioplastics. Oil-based plastics with high biodegradability include PET [27], poly(glycolic acid) (PGA), poly(caprolactone) (PCL), poly(butylene succinate-coterephthalate) (PBST), poly(butylene adipate-co-terephthalate) (PBAT), and poly(vinyl alcohol) (PVA). Note that because the carbon source in oil-based plastics with high biodegradability is petroleum, the carbon moves in one direction from the underground into the atmosphere in the form of CO₂ gas. Biomass-based plastics with low biodegradability include well-known plastics such as bio-PE, bio-PP, bio-polyester, and bio-polyimide. Biomass-based plastics with high biodegradability include the following polymer materials: Starch-based materials [63-65], polylactic acid (PLA) [66,67], polyhydroxyalkanoates (PHA) [66,68,69], polybutylene succinate (PBS) [70], bio-polybutylene adipate terephthalate (bio-PBAT) [71], polyglycolate (PGA), polysaccharide, collagen, bio-PVA [72-75], cellulose, pectin, and chitin. Among these bioplastics, production of PLA and starch-based materials accounts for approximately 10.3% and 18.2%, respectively,

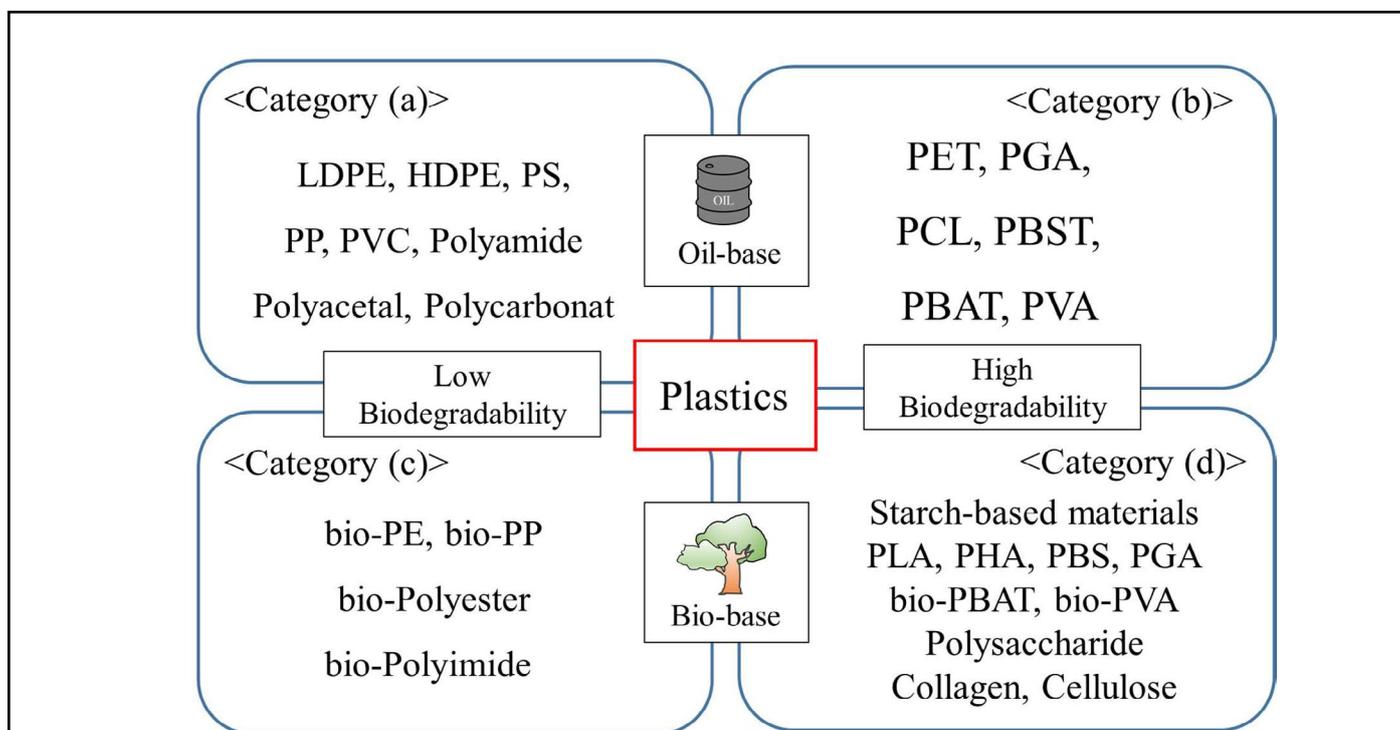


Figure 3: Categorized plastics based on raw materials and biodegradability.

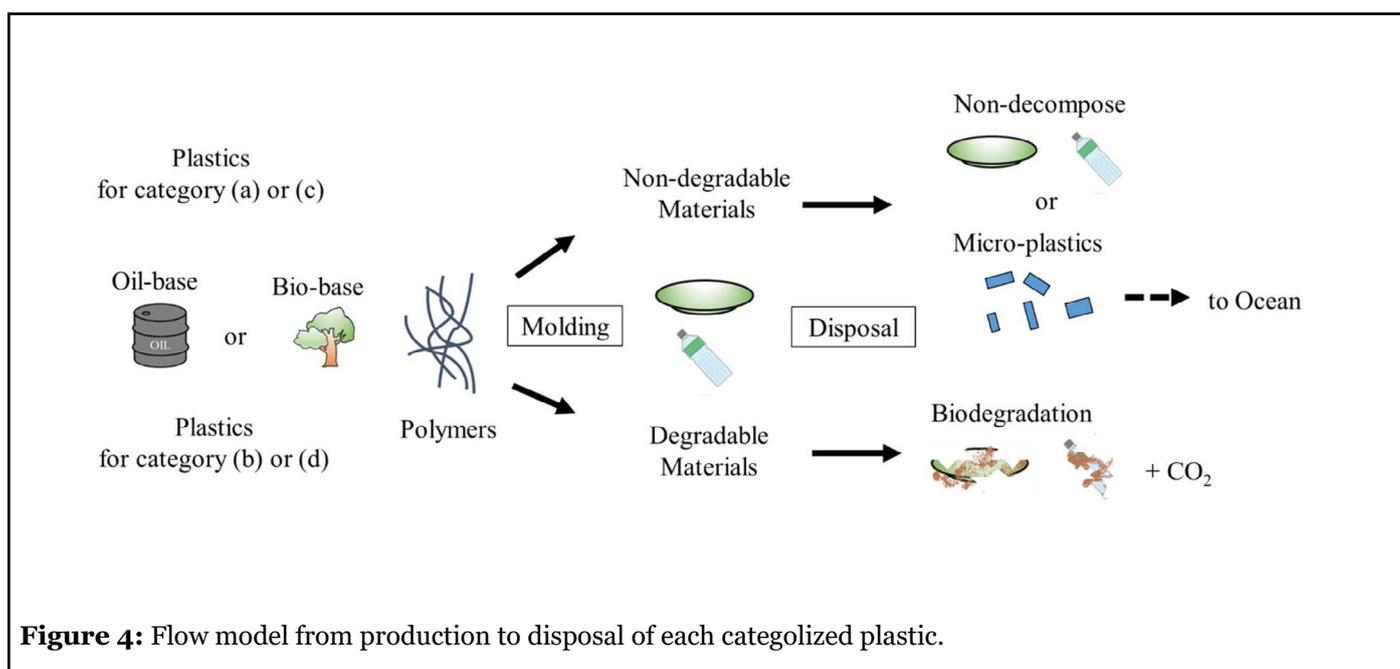


Figure 4: Flow model from production to disposal of each categorized plastic.

of the total amount of the bioplastics in the environment [76] (Figure 4). In addition, because PHAs, PGA, and mixtures of biodegradable polymers are plastics, which are decomposed in a short duration by microbes under suitable conditions, they have already been adopted for practical use [77]. Note that several bioplastics produce fragments with micro- to nano-size owing to biodegradation under unsuitable conditions [78-80], which would affect the environment and human health [81]. In addition to biodegradation, several synthetic polymers show oxidative degradability. Note that plastics with oxidative degradability do not decompose less than the size of microplastics. However, the effect of oxidative degradable plastics on the environment is unclear, unlike that of biodegradable plastics.

To decrease the global environmental pollution due to oil-based plastics with low biodegradability, there have been attempts to produce bioplastics from renewable resources as sustainable materials [38,82]. Bioplastic materials have been produced as packaging and catering products, parts of electronic devices and automobiles, agricultural and gardening supplies, toys, and fibers [56,76]. In addition, they have been used as coating materials for biodegradable films, package supplies, and non-combustible materials as an alternative to oil-based plastics [81]. Bioplastics are widely used in the field of agriculture. For example, they are used as protecting films for seeds. Changing the protecting film from oil-based plastics to bioplastics is advantageous for producing smaller amounts of waste [83-85], with a shorter degradation time and constant degradation rate than those of conventional oil-based films. Moreover, in the case of mixtures of bioplastics and *B. subtilis* spores (a

plant growth promoting bacteria), it was revealed that the rate of biodegradation increased [85]. Overall, research has progressed in this area.

However, in 2017-2018, it was reported that the production of bioplastics was approximately 1% of the total amount of plastics in the environment [76]. Although their producibility gradually increases, it is presumed that the amount of production of the bioplastics will increase from 2.11 million tons in 2018 to only 2.62 million tons in 2023. This is because the production cost of biodegradable plastics is significantly higher than that of the oil-based varieties, although farmers have recognized the importance of alternative plastics for a sustainable society. Further development of biodegradable plastics is required to assess their future social impact.

Anticipation for Designs of Bioplastics as Future Resources

Bioplastics are relatively new materials in various fields, and their use is increasing in the global market.

As described above, bioplastics meet the criteria for bio-based and/or biodegradability. Considering the circular economy based on carbon sources, future preference will be given to bio-based plastics that directly use CO₂ gas available in atmosphere as a carbon source. Research and development have shown some progress in the expansion of bio economics owing to the design of the bioplastics that can degrade into CO₂ over a duration of a few months or years. These were developed based on the carbon circular process. For instance, some bioplastic bottles designed for water storage could retain their form for several years

indoors, whereas bottles of the same materials would degrade in relatively shorter times when in the outside environment. Conversely, the use of bioplastics as rigid resources, including for traffic infrastructure such as road surfaces and building structures, will be preferred because CO₂ can be captured in the resources for a long time. These ideas should involve the production of bioplastics using atmospheric CO₂ as a carbon source. Photosynthetic organisms, including higher plants and microalgae, assimilate CO₂ into organic chemicals as feedstocks for light-energy bioplastics. For example, PHB produced by cyanobacteria could be used as a feedstock [69]. The photosynthetic organisms can grow and produce feedstocks using inorganic substances, including nitrogen and phosphate, suggesting that the produced feedstock can be used as circular resource for carbon and other inorganic materials. Therefore, bioplastics, which are different from oil-based plastics, can be used as organic wastes containing rich nutrients for fertilizers and composts [86-89]. For their use as fertilizers and composts in the environment, degradability, safety, and removal of pollutants and organic waste should be certified [90]. In response to these requirements, several research have reported the results of degradation reactions under aerobic/anaerobic conditions in the environment [86]. Furthermore, discarded PHA can be degraded by microbials and supplied as carbon and energy sources to other organisms [91]. The supplier of feedstock for bioplastics should also consider the cultivating place of photosynthetic organisms to avoid competition for food. Although most bioplastics are currently produced using food-based feedstock such as carbohydrate, achieving SDGs is difficult because of the competition for cropland, water resources, and food supply [92]. Therefore, the system using microalgae, including cyanobacteria and green algae, has the advantages of producing and supplying resources for next-generation bioplastics because these photosynthetic organisms can be cultivated in fresh water and/or seawater and not terrestrial cropland [93,94]. If the production and supply of resources using microalgae becomes a successful strategy, the system will contribute to SDGs.

The competitors in bioplastics market achieve supply by reducing production costs and increasing quality. Therefore, the process of extracting and fractionating components of bioplastics from biomass has already been practiced for a long time [95]. For instance, the mechanical cracking method for cells and processes of using supercritical water have been extensively studied. Therefore, efficient extraction and fractionation of carbohydrates, lipids, proteins, nucleolus, and cell walls have been improved. These approaches have exhibited the possibility for realization of bioplastics without the high costs of fractionation. Furthermore, progress in the CRISPR technology used on microalgae can lead to the

optimization of light-harvesting efficiency and installation of novel metabolic pathways in the cells [96,97]. Genetic engineering for improving specific metabolic pathways allows the possibility of increasing the productivity of value-added materials using microalgae. Although the GMOs require particular attention to deter them from escaping externally, the use of GMOs can result in an efficient process and reduction of production costs. Especially, the production of value-added substances using microalgal GMOs can resolve the problem of high cost. In addition, the parallel production of resources for bioplastics from the same biomass can result in reduced cost.

For biodegradable plastics, it is necessary to set suitable conditions for biodegradation. Under unsuitable conditions, small pieces, such as micro- or nano-plastics, can be produced and get accumulated in the environment before the complete biodegradation of bioplastic. Microplastics that originated from bioplastics affect the ecosystems at coastal waters and on the deep seafloor [29,98], similar to oil-based plastics [29,98-101]. For example, when PLA is accumulated on the deep seafloor, the *Ostrea edulis*, known as the flat oyster and *Arenicola marina* L [98], become stressed and their breathing rates increase. The exposure of microplastics, namely PHB and polymethylmethacrylate, which is a petroleum-based plastic, to *Gammarus fossarum* resulted in a reduction in its assimilation [99]. Moreover, in the case of *Anabena* sp. PCC7120 and *C. reinhardtii*, significant reduction in their cell proliferation and modifications in relevant physiological parameters were found. Conversely, for the crustacean *Daphnia magna*, the induction of immobilization and significant increase in intracellular reactive oxygen species levels were investigated [100]. Serious membrane damage in these three organisms was reported [100]. Other reports revealed that mortality increased, whereas the feeding rate and fertility decreased in the case of *D. magna* exposed by microplastics of PS [102]. In comparison with the oil-based microplastics, biodegradable plastics could be a vector that injects pollutants into the body [101,103-107], thereby suggesting that plastics dispersed in the environment have a negative impact on the ecosystem, including bioplastics. There are several unclear points in the biodegradation process of the microplastics of bioplastics [28,108]. Given the recent development in the field of bioplastics, there is a gap between the environmental impacts of microplastics and our findings.

Studies have been conducted on the overall process of degradation, such as end-of-life options [109], photolysis and thermal oxidative degradation [110-112], and biodegradation [111,113-115] to understand the degradations of the bioplastics that are designed to break down into small molecules, (e.g., monomers,

dimers, and oligomers) in the environment. Recently, utilization of microbial strains involved in the degradation process has been evaluated because microorganisms decompose the organic substrate of the bioplastics [115]. To understand the degradation process, biodegradation process under dry/wet or aerobic/anaerobic conditions have been tested. Real-life tests directly evaluate the decomposition of bioplastics in the environment [116,117]. Biodegradation of bioplastics is evaluated based on (ultimate) biodegradability, disintegration, and compost quality [28,111,117]. Biodegradability is the ability of bioplastics to be broken down into small molecules by both biological and abiotic effects during hydrolysis process. Disintegration means physically crushing substances to small pieces [118]. The compost quality is evaluated by measuring the weight of microplastics with sizes more than 2 mm, which is found by sieving of compost [119-121]. Note that to the best of our knowledge, there is no protocol for monitoring plastics with size less than 2 mm. The information on the effects of test substances is provided through environmental toxicity tests conducted in the cultivated land [113]. Moreover, degradation in several different types of bioplastics was evaluated for the contribution of the physical and chemical properties of bioplastics to biodegradation [92,105,115,122], in addition to the conditions of the production process. Note that physical and chemical properties, which are considered for biodegradation in the environment, are hydrophilicity, surface area, molecular weight, chemical structures, and higher order structures [122]. During the biodegradation process, plastic fragmentation proceeds by physical process, such as polishing power, heating/cooling, freeze/thaw, and wet/dry, and chemical process, such as oxidation and hydrolysis [123,124]. In the case of biodegradation in the environment, several microbial enzymes such as depolymerase, lipase, cutinase, hydrolase, protease, and lignin modifying enzyme cleave polymers to oligomers, dimers, and monomers [125]. Fragments pass through the cell wall of microorganisms; therefore, they are eventually decomposed into carbon dioxide when they are used as a substrate for microorganisms [115]. Comprehensive analysis shows that monomers belonging to the metabolic processes of cells produce energy that cause decomposition into water, carbon dioxide, biomass, and other basic products through various metabolic and enzymatic mechanisms [125]. Therefore, when the bioplastic is made of raw, recyclable materials that are universally present in environment, they would be treated as eco-friendly materials because they would be easily decomposed in the environment and be ingested by organisms.

Cell-plastics as Neo-plastics

As described above, the use of bioplastics is not wide spread yet owing to their costs. Thus, the production of a

new circular resource, which can be serve as an alternative to the oil-based plastics, is crucial. Recently, a novel bioplastic made of green algal cells was reported [126]. *Chlamydomonas reinhardtii* is a unicellular green alga that self-propagates using CO₂ as a carbon source [127]. *C. reinhardtii* can be used as an industrial strain as follows owing to the good proliferative productivity (10–50 times higher assimilating activity of CO₂ than that of popular terrestrial plants [128]), properties producing value-added materials such as lipids and carotenoids [129,130], and bio-safety with no reports about toxicity [131]. The cells are significantly rigid, and therefore, the breaking cells that extract intercellular materials require powerful breaking methods, such as beads beater [132]. Although the rigidity and robustness of *C. reinhardtii* cells are the bottlenecks of extracting the intercellular components, its tough physical properties make it a potential candidate to be used as an ingredient in plastics. In the case of using unicellular *C. reinhardtii* as a plastic resource, cells can be freely placed on empty spaces; however, cells require a filler to connect to each of the cells. In this study, glycerol and bovine serum albumin were used as filler, which connected the cells to each other, thereby constructing a cell-layer. The cell-layer was expected to be used as a plastic resource because of the possibility to be freely molded using templates and was referred to as “cell-plastics” [126]. As the cell-layer is a fragile material because of the weak interactions between the cells, it is necessary to improve mechanical strength for actual use. Therefore, stacked structures of cell-plastics were constructed by alternatively stacking the cell-layer and flexible thin organic film such as a two-dimensional polymer, which has a sheet-like structure with a two-dimensional periodicity [133-136]. A self-standing film of the cell-plastic was thus manufactured, which was not realized only with the cell-layer (Figure 5). The plastic was composed of green algal cells, without the extraction of intercellular components and was produced as a next-generation bioplastic, possibly responding to the SDGs for the first time. The considerable research accomplishment is to be followed by progressing up to the next stage, wherein the cells could be used as direct ingredients of plastics with reduced costs. In future, cell-plastics that are durable in actual use should be fabricated using biodegradable fillers that have a good affinity with the cells via chemical or supramolecular interactions; furthermore, a carbon-recycling system should be achieved.

Bioplastics exhibit high potential as an alternate for oil-based plastics, although they can damage the environment and biogeocenosis, similar to oil-based plastics, if their production and plastic waste disposal are ignored. However, there is no doubt about the future breakthrough of bioplastics, and we believe that production and discard of bioplastics will be considered sufficiently in the future.

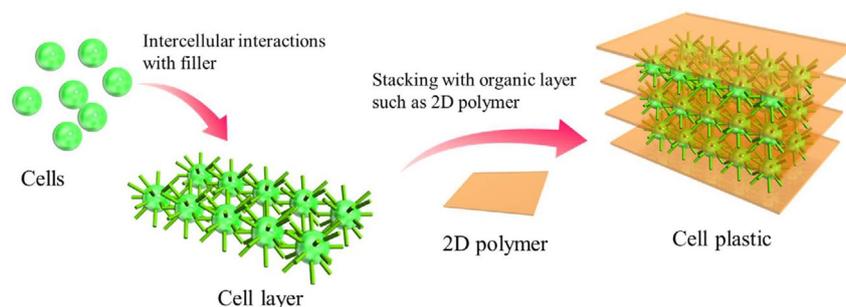


Figure 5: Schematic model of fabrication of cell-plastics.

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