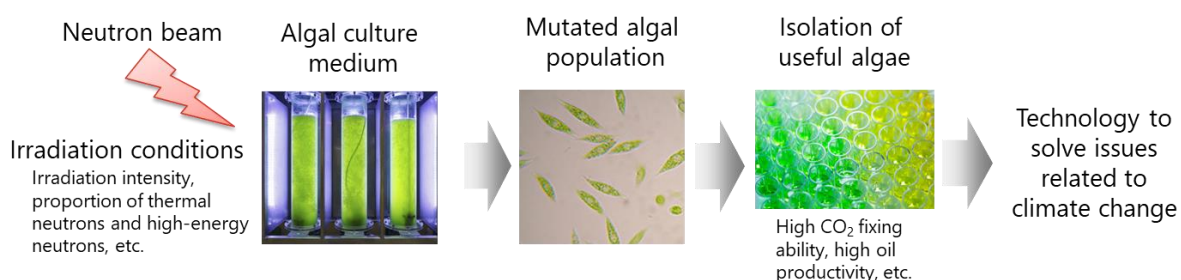


## Start of demonstration experiment of algal breeding technology to solve climate change issues

–Aiming to establish the world’s first useful algae breeding technology by introducing genetic mutation through neutron irradiation–

Nippon Telegraph and Telephone Corporation (NTT, President & CEO: Akira Shimada) and Euglena Co., Ltd. (Euglena, Founder & President: Mitsuru Izumo) have started a demonstration experiment of algal breeding technology using neutron beam\*<sup>1</sup> irradiation aimed at solving issues related to climate change, such as reducing greenhouse gas emissions and creating energy resources.

The technology to be tested in this demonstration experiment is a technology to enhance useful traits\*<sup>2</sup> of algae, such as their ability to absorb and fix CO<sub>2</sub> (carbon dioxide) and produce oil as feedstock for renewable fuels, by introducing genetic mutations\*<sup>3</sup> through neutron irradiation. Neutron beams are extremely penetrating compared to other types of radiation, and can add energy to organisms such as algae, which need to be cultured in solution, in an irregular and effective manner. The ability to introduce various genetic mutations by appropriately selecting and irradiating with thermal and high energy neutrons makes it possible to breed and produce algae with enhanced usefulness according to the purpose of use. This technology is expected to solve various issues related to climate change, including not only the reduction of greenhouse gas emissions and the production of energy resources, but also the creation of food resources and feeds for agriculture, forestry, and fishery (Fig. 1).

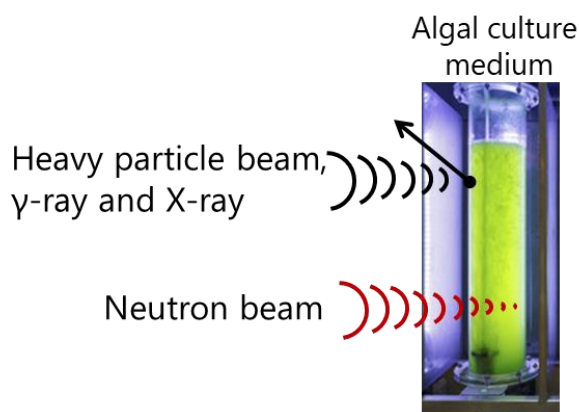


**Fig. 1. Overview of this demonstration experiment**

## 1. Background

According to the Intergovernmental Panel on Climate Change (IPCC) Sixth Assessment Report (<https://www.ipcc.ch/>), it is stated that “It is unequivocal that human influence has warmed the atmosphere, ocean and land”. Thus, there is an urgent need to reduce greenhouse gases such as CO<sub>2</sub> emitted by human activities. Algae produce organic matter and proliferate by absorbing and fixing CO<sub>2</sub> dissolved in water through photosynthesis. Therefore, algae cells themselves and organic matter produced by algae can contribute to CO<sub>2</sub> reduction and can also be utilized as energy and food resources, and algal breeding with useful traits is attracting attention as a means of solving various issues related to climate change.

Breeding methods that promote genetic mutation in algae have been studied using electromagnetic waves such as  $\gamma$ -rays and X-rays, as well as heavy particle beams<sup>\*4</sup> possessing electric charges. In these breeding methods, high energy electromagnetic waves or heavy particle beams are used to induce some mutation in the gene sequence, and cells with the desired traits are selected from the mutated cell population. However, because electromagnetic waves and heavy particle beams have low permeability through materials, irradiation of algae growing in a culture medium has been problematic since only some cells on the surface of the medium are irradiated and many other cells in the medium are unaffected (Fig. 2).



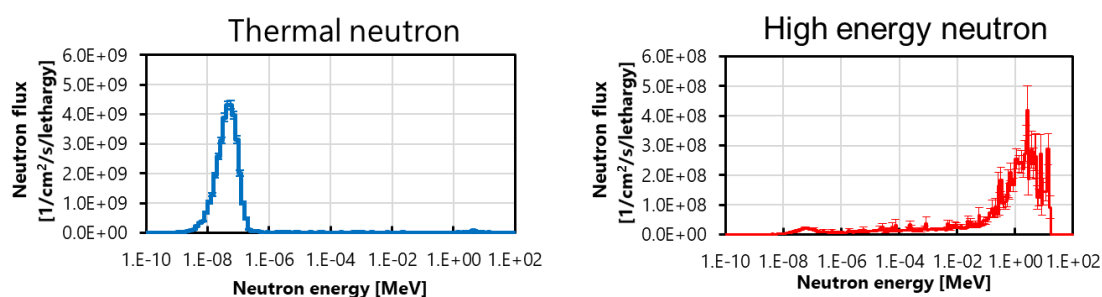
**Fig. 2. Types of radiation and differences in penetrating power**

## 2. Technology features and demonstration models

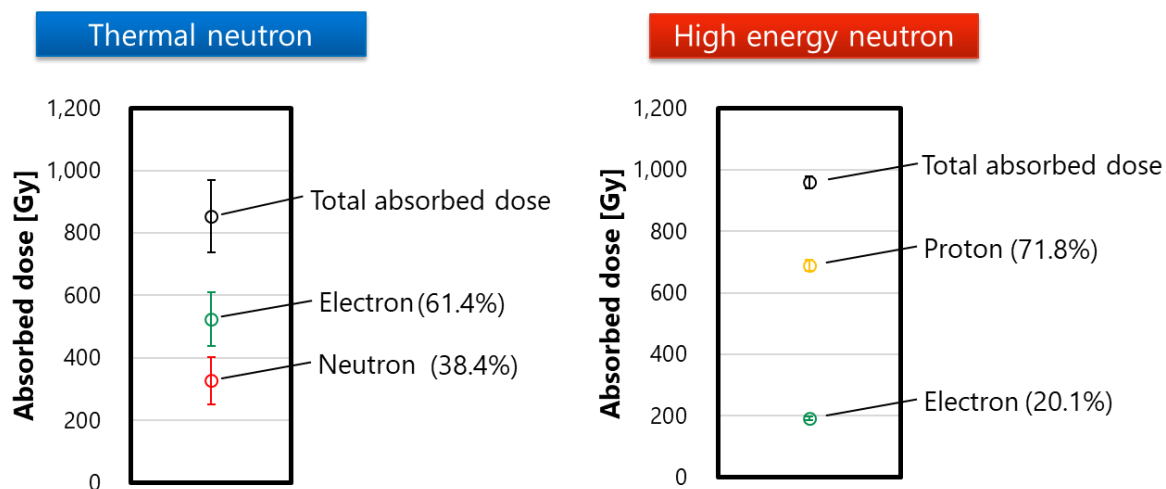
In this demonstration experiment, we aim to establish a breeding method that enables various trait changes and efficient gene mutagenesis by irradiating all algal cells in the culture medium in an irregular manner using neutron beams, which have no electrical charge and a high ability to penetrate matter. Although the feasibility of genetic mutagenesis of algae has been confirmed in previous studies using neutron beams, it has not yet been established as a breeding technology, including the preconditions for inducing mutations and the selection of good strains after irradiation. We will attempt to clarify the optimum irradiation conditions for each breeding objective and to optimize the process of the breeding.

In neutron irradiation, in addition to fast-moving high energy neutrons, slower thermal neutrons can be used as the main component (Fig. 3). A simulation result conducted by NTT predict that the type of particles that contribute to the absorbed dose\*<sup>5</sup> that induces genetic mutation in algae differ between thermal neutrons and high energy neutrons (Fig. 4). When algae in the culture medium are irradiated by thermal neutron beams, they are mainly affected by electrons produced by the reaction of thermal neutrons and water molecules. In contrast, when algae are irradiated with high energy neutrons, they are mainly affected by protons produced by the reaction of high energy neutrons and water molecules. We will examine different effects of the two types of neutrons, which act differently in the culture medium, on the genetic mutation in algae.

NTT has demonstrated that neutron rays generated when cosmic rays\*<sup>6</sup> collide with oxygen and nitrogen in the atmosphere cause soft errors\*<sup>7</sup> in semiconductors of communication devices used on the ground, and has contributed to the formulation of ITU-T\*<sup>8</sup> that describes its evaluation methods and countermeasure. Currently, NTT is conducting research on technologies to prevent the human body and electronic equipment from being affected by cosmic rays in space. The know-how on neutron irradiation accumulated in the process will be applied to neutron irradiation against algae cells, and NTT will be in charge of the quantitative evaluation of the effects. Euglena will be in charge of isolating and characterizing cells that show useful traits from the cell population after neutron irradiation utilizing the technologies that can evaluate cells suitable for producing oils that can be used as feedstock for renewable fuels and the functional material, paramylon\*<sup>9</sup>. Through these verifications, we can optimize algae culture conditions, neutron irradiation conditions, and the breeding and selection process for the desired trait change, and then aim to establish breeding techniques for algae with traits suitable for various purposes and with a high level of safety.



**Fig. 3. Spectra of thermal and high-energy neutrons**



**Fig. 4. Types of particles that contribute to the absorbed dose of thermal and high-energy neutrons**

### 3. Future perspective

NTT and Euglena will conduct a series of demonstration experiments of algae breeding technology on several species of algae using their growth rate, ability to absorb and fix CO<sub>2</sub>, and productivity of oils as indicators, with the aim of realizing the technology. We will also examine methods for effectively selecting useful strains suitable for various purposes. In the future, we will develop this technology into a highly versatile breeding technique that can maximize organisms' potential, including targeting not only algae but also microorganisms that are useful in solving climate change issues.

\*1 Neutrons are particles that make up the nucleus of an atom. When an atomic nucleus undergoes nuclear fission, neutrons are ejected with kinetic energy out of the nucleus. These neutrons moving in one direction are called neutron beams.

\*2 Trait is a characteristic or attribute of an organism.

\*3 Genetic mutation indicates a change in the gene sequence from its original sequence. As a result of a genetic mutation, the function of the protein produced from the gene is altered.

\*4 Heavy particle beams are particle beams that are heavy mass. Particle beams such as helium, carbon, neon, and argon classify into this category.

\*5 Absorbed dose is the energy absorbed by a material due to irradiation, and here it denotes the measure of the effect of radiation on the cells.

\*6 Cosmic rays are high energy radiation that travels through space and are mainly composed of protons, but also include alpha particles, lithium, beryllium, and other atomic nuclei. In space, cosmic



rays can affect electronic equipment and the human body. On the ground, neutrons produced by cosmic rays reacting with the Earth's atmosphere can cause electronic equipment to malfunction in rare cases.

\*7 Soft error is a temporary failure that is recovered by restarting the device or overwriting data, unlike hard errors, which cause permanent device failure.

\*8 ITU-T is an abbreviation of the International Telecommunication Union-Telecommunication sector.

\*9 Paramylon is a polysaccharide produced by the Euglena genus as an intracellular storage material and is a type of dietary fiber. Recent research data have shown that it has new functions in health care that are different from conventional dietary fiber, such as its effect on immune function.

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