## Genetic engineering for establishing biohydrogen production technology

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## (1) Biohydrogen production

Hydrogen fermentation is divided into light-independent dark fermentation and light-dependent photofermentation. This biological hydrogen production technology offers the advantage of operating at ambient conditions, resulting in minimal environmental impact. Furthermore, it enables the development of a zeroemission process through CO2 recycling, using biomass as the feedstock. Our research group, in collaboration with Sharp Corporation, has achieved a high-speed hydrogen production process utilizing a dark fermentation hydrogen production pathway involving formic acid. The hydrogen production rate achieved by our process is one to two orders of magnitude higher than that of conventional fermentation processes. On the basis on this achievement, we are working on improving hydrogen yield by introducing heterologous hydrogen-producing enzymes (hydrogenases) through genetic engineering. This advancement allows for the construction of novel hydrogenproducing microorganisms capable of producing up to four moles of hydrogen from one mole of glucose. Additionally, we are engaged in technology development aimed at establishing an integrated process with photofermentation, theoretically enabling the production of up to 12 moles of hydrogen. (2) Liquid biofuel production

Our research group has been advancing the development of metabolic engineering technologies using coryneform bacteria, industrially valuable microorganisms with a long history of application in amino acid production. In conjunction with this, we have also developed a proprietary growth-independent bioprocess known as RITE Bioprocess. The combination of these technologies enables the highly efficient utilization of nonedible biomass-derived sugars, establishing a high-yield bioprocess that demonstrates significant advantages in terms of fermentation inhibitor tolerance and simultaneous utilization of mixed sugars. Building upon these foundational technologies, in this project, we are constructing an ethanol production process from a wide range of non-edible biomass feedstocks and obtaining proof-of-concept data to move towards practical applications.

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