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学 位 論 文 内 容 の 要 旨

博士 (環境科学)

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学 位 論 文 題 名

A trial to enhance the production yield of a duckweed, *Lemna gibba*,
in food factory wastewater utilizing indigenous bacteria

(食品工場排水における土着細菌を活用したウキクサ植物 *Lemna gibba* の生産収率向上の試み)

The Duckweed, Lemnoideae plants, are recently highlighted as one of the useful biomass with multiple uses. The development of effective production of the duckweed biomass using wastewater as cost-free fertilizer is the challenge of this study. In this study, I conducted a trial to grow a duckweed *Lemna gibba* in the treated food factory wastewater (WW) from Thailand, namely A-wastewater (A-WW) and K-wastewater (K-WW). The WW contained significantly higher sodium (Na) and phosphate (PO_4), while lower nitrogen (N) by 100-folds compared to a popular duckweed medium, Hoagland.

Plant growth-promoting bacteria, PGPB, are currently expected to become a new nature-based technology for increasing biomass production such as the duckweed. In chapter I, I call attention to the use of appropriate PGPB, especially in A-WW and K-WW with uneven nutritional conditions. Highly active PGPB, *Acinetobacter calcoaceticus* P23 and *Pseudomonas fulva* Ps6, previously obtained from the environmental water in which duckweed naturally grows, did not promote but instead inhibited the growth of *L. gibba* in the WW. Thus, I explored indigenous bacteria from the WW that exhibited growth promotion activity towards *L. gibba*. I demonstrated for the first time that among the indigenous bacteria that naturally grow in the WW unrelated to duckweed habitat, there exist a specific group of bacteria that can promote the growth of *L. gibba* in both WW conditions. Moreover, I determined key factors caused the growth inhibition activity of the above mentioned non-indigenous PGPB strain P23, in the K-WW.

Nevertheless, there is still a challenge remained of the PGPB application in WW, where the biomass yield of *L. gibba* could not fully recover. The WW contained the unbalanced minerals that significantly reduced *L. gibba* growth and also affected PGPB activities. Thus, in chapter II, I further identified factors for growth inhibition of *L. gibba* in the WW and demonstrated that modification of WW improved the growth of *L. gibba* and PGPB activities. Supplementation of N and CaCO_3 application to the WW resulted in the recovery of *L. gibba* growth. The results indicated that low N and excess PO_4 were the crucial factors that limited the growth of *L. gibba*. Moreover, all the PGPB,

including non-indigenous P23, showed higher duckweed growth promotion activities in the mineral-modified WW. Overall, the mineral supplementation combined with the utilization of domestic PGPB offers a promising way to improve duckweed biomass production in the WW significantly.

On the other hand, during the trial of wild duckweed biomass production in an open-air A-WW tank, Thailand, we observed the overgrowth of microalgae, which dramatically reduced the duckweed growth probably because of their nutrient competition. Therefore, in chapter III, I explored the possibility whether the indigenous PGPB for *L. gibba* also have antagonistic activity towards the microalgae. Some eukaryotic microalgae were isolated from wastewater samples, including genera *Chlorella*, *Coelastrella*, *Desmedesmus*, and *Parachlorella*. In addition, *Microcystis aeruginosa*, a prokaryotic microalga well-known to form harmful algae bloom (HAMs) by producing microcystin toxin, was also used for examination. It was found that some PGPB have the ability to inhibit the growth of microalgae, especially *M. aeruginosa*. This result strongly suggests that we have a chance to discover further wonderful PGPB which have a dual function to enhance the duckweed growth while inhibiting the growth of duckweed's competitor, microalgae. Overall, this study uncovered the hidden potentials of indigenous PGPB that contribute to improve the production efficiency of the duckweed biomass using factory wastewater. In order to put the strategies developed here into practical use, it is needed to evaluate feasibility of the scale-up duckweed cultivation system in the actual WW treatment sites for future.