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論　文　の　要　旨
Abstract of thesis

Food safety is a vital concern in food preservation. In general, foods are processed by intervention technologies to inactivate microbes. However, bacterial injury after sublethal inactivation should be paid more attention, since injured bacteria are still viable and may recover to regain their ability for proliferationduringstorage.Bacterial injury and recovery might result in overestimation of food safety, being high risks in the food industry.In this study, *Escherichia coli* ATCC25922 was adopted as a model bacterial strain, and two sublethal stresses, hyperosmotic NaCl and High Hydrostatic Pressure (HHP), were investigated individually or synergistically to clarify the bacterial viability, injury and recovery. Subsequently, colony formation of the HHP-injured cells wasevaluated by image analysis to reveal the different levels of bacterial injury.

In Chapter 1, the author reviewed the background information in terms of bacterial inactivation, injury and recovery, NaCl effects,HHP pasteurization, hypothesis on levels of bacterial injury and colony formation of injured cells.

In Chapter 2, the author studied the effect of hyperosmotic NaCl and storage temperature on healthy *E. coli* cells. Cells of *E. coli* were suspended in phosphate-buffered solutions containing NaCl of 0.9, 3.5, 5.0, and 10.0 % and stored at 5, 10, 15, 20, 25 °C. Results demonstrated that the effect of NaCl on the cells varied, depending on the storage temperature. Hyperosmotic NaCl slightly protected the cells at 5 and 10 °C, while enhancing inactivation with increased storage temperature and NaCl concentration. Cells were inactivated from ca. 9 log CFU/ml (hereafter, log) to nondetectable level in 10.0 % brine, and possible adaptation was observed at NaCl ≤ 5.0 % at 20 and 25 °C, leading to a final cell counts of ca. 6 log. Injured cells were observed limitedly in 5.0 and 10.0 % brines. However, the injured cells recovered in 5.0 % brine by 2 log cycles during storage at 20 °C.

In Chapter 3, the author investigated the effect of hyperosmotic NaCl and storage temperature on the injury and recovery of HHP-treated *E. coli*. After HHP treatment (400 MPa, 25 ºC, 10 min), the total viable cell counts decreased from ca. 9 log to ca. 2 - 4 log, and all or most of the viable cells were judged to be injured. Healthy cells were not detected in 0.9 % brine after HHP treatment, while the healthy cell counts were detected higher in higher-concentration brines (0.47 log, 1.20 log, and 2.32 log in3.5 %, 5.0 %, and 10.0 % brines, respectively). It was indicated that NaCl protected the cells from the HHP inactivation slightly. On the other hand, the recovery of injured cells to ca. 8 log was observed in 0.9 % brine during storage at 15, 20, and 25 ºC but not in the other brines, indicating either refrigeration temperature (≤ 10 ºC) or hyperosmotic NaCl (≥ 3.5 %) suppressed the recovery. Furthermore, it was suggested that the synergistic treatment of medium HHP and low-concentration ofNaCl could inactivate the *E. coli*cells efficiently.

In Chapter 4, the author studied the different levels of injury after HHP treatment, which might affect the subsequent bacterial recovery, from the heterogeneous colony formation.In this study, healthy *E. coli* cells were treated with HHP of 200, 300, and 400 MPa to obtain injured cells at different stress levels, and colonies were analyzed using the software "ImageJ®". In order to quantify the colony formation, time distribution of colony emergence, equivalent circle diameter, and relative frequency of colonies were defined and analyzed. Viable cell counts of 10.34 ± 0.08 log decreased to 10.08 ± 0.04 log, 7.13 ± 0.01 log, and 4.69 ± 0.01 log after 200, 300, and 400 MPa treatment, and healthy cells of 10.38 ± 0.03 log decreased to 9.28 ± 0.02 log, 3.13 ± 0.04 log, and 1.32 ± 0.37 log, respectively, indicating most of the *E. coli*survivors were injured. All colonies of the untreated cells emerged after 1 d incubation, whereas 17.9 % and6.0 % of the 400 MPa-treated cell colonies emerged after 4 d and 5 d incubations. Moreover, the average colony diameter decreased as HHP increased. However, the decreasing trend became less-pronounced after 2 d incubation probably due to the increased effect of colony density, indicating the limits of this method. Broad distribution curve of colony diameter after 400 MPa treatment suggested the heterogeneity of bacterial injury. As compared with colony diameter and relative frequency, the time distribution of colony emergence clearly presented the bacterial injury.

In Chapter 5, the author summarized the major conclusions from the whole study and prospected future researches.

審 査 の 要 旨
Abstract of assessment result

In this dissertation, two sublethal stresses, hyperosmotic NaCl and HHP, were investigated individually or synergistically to reveal the bacterial injury and recovery. In order to judge the injured bacteria, selective and nonselective colony counting medias were applied simultaneously. In terms of the different levels of bacterial injury, colony formation of the HHP-injured cells was evaluated by image analysis. Through this study, the author clarified: 1) the viability, injury and recovery of *E. coli* cells under hyperosmotic stress, 2) injury and recovery of *E. coli* after HHP treatment in isosmotic system, and 3) injury and recovery of HHP-treated *E. coli*in hyperosmotic systems. Results from this study implicated that both hyperosmotic NaCl and HHP stresses were sublethal on the inactivation of *E. coli* due to the possible adaptation to NaCl (≤ 5.0 %) or the recovery of injured cells after HHP treatment (in 0.9 % brine, 400 MPa). When combining these two treatments, HHP induced the injury of *E. coli* cells, meanwhile hyperosmotic NaCl suppressed the viability of HHP-injured cells even at 3.5 % NaCl. It was also indicated that HHP treatment could contribute to the reduction of NaCl addition in food preservation, suggesting a feasibility of synergistic treatment of HHP and low concentration of NaCl as an intervention technology in food processing. In addition, the image analysis on colony formation provided a new vision to evaluate the levels of HHP-induced bacterial injury, contributing to the work on analyzing the injured cell populations more in detail.

The final examination committee conducted a meeting as a final examination on 14thJanuary, 2020. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

Therefore, the final examination committee approved that the applicant is qualified to be awarded the degree of Doctor of Philosophy in Environmental Studies.