論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Establishment of gas fermentation platform combined with water electrolysis for production of value-added chemicals from CO2 and evaluation of engineered Ralstonia eutropha
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出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第12751号, 授与年月日:2024年3月26日, 学位の種別:課程博士, 審査員:福居 俊昭,蒲池 利章,平沢 敬,松田 知子,柘植 丈治
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第12751号, Conferred date:2024/3/26, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 生命理工学	系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of	（工学）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Global warming caused by the increase of atmospheric greenhouse gases and the persistent presence of plastics and microplastics in the ocean is a persistent problem that scientists are trying to solve. Biotechnology is playing a growingly important role in the business strategies of several countries, yet finding a consistent and sustainable feedstock of carbon that does not compete with the food supply is one of the most difficult challenges to overcome. The direct utilization of CO_2 in biotechnological processes has been attracting strong interest in the scientific community in recent years.

"Knallgas" H_2-oxidizing bacteria are possible candidates for this purpose because they show fast growth on CO_2 regardless of light irradiation. *Ralstonia eutropha* is a kind of the H_2-oxidizing bacteria and has been known to be an efficient producer of polyhydroxyalkanoates (PHAs). In contrast to many studies on this bacterium focusing on heterotrophic production of PHAs and other chemicals, detailed investigations regarding aerobic gas fermentation with this bacterium have been limited mainly due to the hazardousness of explosive mixture of H_2 and O_2, resulting in poor knowledge about the gas fermentation. The use of a safe, cost-effective gas mixture that allows for the efficient and sustainable production of chemicals could be an elegant and functional solution to the above issues.

This study investigated the ability of *R. eutropha* to utilize safe gaseous mixtures of H_2, CO_2 and O_2 to produce PHA copolymer applicable as biodegradable plastics, and alcohol as an industrial chemical as well as biofuel. In particular, the investigation focused on yields of the products on input H_2 and electricity as well as the bioconversion efficiencies, because the H_2 supply will occupy most of the raw material cost in bioproduction by H_2-oxidizing bacteria.

A novel recycled-gas closed circuit (RGCC) culture system with a non-combustible gas mixture, in which the H_2 concentration was lower than the explosion limit, was developed. H_2 and O_2 were supplied from water by a proton exchange membrane-type water electrolyzer with a membrane electrode assembled with a platinum/carbon cathode and manganese-iridium composite oxide anode. The gas flows were managed by automated feedback regulation, allowing constant gas composition throughout the operation and determination of the product yields and biosynthesis efficiencies on H_2 as well as electricity. The autotrophic cultivation of the engineered strain of *R. eutropha* using the RGCC culture system demonstrated the production of P(3HB-*co*-12.5 mol% 3HHx) from CO_2, with the yield of 63% on input H_2 and electricity-to-PHA efficiency of 10.7 %. These results showed the high potential of *R. eutropha* to produce useful compounds from CO_2.The effects of genetic modifications in *R. eutropha* aiming to enhance the ability to grow and produce PHA from the gaseous substrates were investigated as well. The introduction of exogenous *can* encoding cytosolic carbonic anhydrase increased the molar fraction of 3HHx in the resulting copolyester up to 19.6 mol%, probably due to higher availability of CO_2 for crotonyl-CoA carboxylase/reductase in the biosynthesis pathway. The RGCC culture system was then applied for autotrophic production of alcohol. The *R. eutropha* strain engineered for alcohol biosynthesis from glucose was demonstrated to produce bioalcohol also from CO_2.

Taken together, the developed RGCC culture system enables not only the bioproduction of PHA and alcohol from CO_2 and electricity but also the evaluation of the bacterial performance in the autotrophic processes. The results suggested loss of input H_2 during the fermentation, and energy loss in the bacterial metabolisms. Interestingly, *R. eutropha* exhibited high CO_2 fixation efficiency in the growth phase, whereas the efficiency was significantly reduced during the production phase. This suggested the presence of some energy-consuming

metabolisms and/or lower efficiencies of energy-conserving metabolisms in the cells after the growth, so the elucidation of the reason for this phenomenon and establishment of modifications to maintain the high CO_2 fixation efficiency even in the production phase could be an interesting and important issue. Engineering of machinery for increasing uptake and conversion of gaseous H_2 and O_2 as well as carbon metabolisms started from the inorganic carbon could be also important to improve the cell growth and biosynthesis of target compounds.