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主論文の要約

博士の専攻分野の名称：博士（水産科学）　　　　　　氏名：Nurhidayu binti Al-saari

学 位 論 文 題 目

A strategic approach for optimization of fermentative hydrogen production of marine *Vibrionaceae* species using Design of Experiments (DOE)

（実験計画法に基づく海洋性ビブリオの水素生産の最適化）

All fossil fuels will inevitably be depleted and their current use is negatively affecting the environment. Hydrogen gas (H_2) is considered to be an ideal alternative global energy source, allowing us to reduce our dependency on fossil fuels. Currently, however, the majority of hydrogen production processes are still heavily dependent on the reformation of fossil fuels. Among these processes, biological H_2 (bioH_2) production offers the best option satisfying both "renewability" and "sustainability" as an alternative energy source. This process utilizes various biological organic substrates obtained from cellulosic biomass of agricultural residues and food industries, carbohydrate-rich industrial wastewater, and domestic wastewater sludge. Numerous feedstock choices have alleviated public concern over the "food vs. fuel" debate and it is now more economically and environmentally sustainable.

Macroalgae-derived biofuel is currently the subject of much discussion as a potentially sustainable and renewable alternative energy source to fossil fuels. Marine macroalgae are known to predominantly contain unique carbohydrates, but are also rich in minerals and salts compared to terrestrial plants, prompting further development of new and optimized technologies for practical biofuel production using macroalgae as a feedstock. In particular, the use of microorganisms that can efficiently convert macroalgae-derived sugars in saline conditions could offer major advantages. *Vibrio tritonius*, a marine bacterium, has previously showed the ability to produce bioH_2 from mannitol and glucose as a substrate under saline conditions via its fermentation pathway. Such BioH_2 production via dark fermentation shows huge potential for biofuel production as it occurs under a simple operating system and offers a high production rate. Fermentation, however, is a progressive process involving many factors that could have independent and/or mutual impacts on production. So, in this study, I aim to find and develop an optimized operating system for the fermentative bioH_2 production of *V. tritonius* AM2 on the basis of a strategic approach.

Optimization of such complex processes requires an approach which allows the understanding of the kinetics of process, observation of the effects of many factors simultaneously, elucidating the relationship among those factors and finding the optimal condition that are feasible to the responses investigated. In this thesis, I used "Design of Experiment (DOE)" complemented with kinetic models for a more strategic and accurate optimization. A modified Gompertz model described the H_2 production and the cell growth, while a Monod model explained the inhibitory effects of Na^+ concentration on H_2 production. One-factor-at-a-time (OFAT) design was first used to elucidate the effects of initial Na^+ concentration on H_2 productivity. The productivity was measured by three

responses; molar H_2 yield, H_2 production rate (HPR), and substrate conversion efficiency (SCE). *V. tritonius* achieved the most optimal productivity when grown in media supplemented with 1.0% (w/v) NaCl concentration. Our data suggests that AM2 is capable of producing H_2 under a wide range of NaCl concentrations up to 4.6% (w/v) NaCl (C_{crit}). The HPR and SCE decreases as the NaCl increases but the concentrations are high enough to nominate AM2 as a good candidate for macroalgae-derived bioH_2 production.

Secondly, in Chapter 3, I used a faced-centered central composite design (FCC) with response surface methodology (RSM) to investigate the effects of pH, substrate concentration and inoculum size and their interaction on H_2 productivity of AM2. The design and models applied have helped discover the correct optimum conditions for H_2 production of *V. tritonius* at 3.0% (w/v) mannitol at pH 5.8 with 0.5% (v/v) pre-cultured inoculum of 0.8 to 1.0 ± 0.1 OD_{620}. Fermentation under optimal conditions resulted in 29.0 HPR, yield of 1.6 mol H_2/mol mannitol and 100% SCE which equivalent to 100%, 14% and 42% increase in HPR, yield and SCE, respectively, than the original condition at 2.25% (w/v) NaCl, 5.0% (w/v) mannitol, pH 6 with 1.0 mL inoculum. RSM showed significant effects of pH, substrate concentration, and inoculum size on hydrogen productivity. HPR was increased as three factors increased, while yield and SCE decreased at higher substrate concentration and inoculum size. Strong interaction of pH and substrate concentration is present in this system.

Formate hydrogen lyase (FHL) complex is reported to be major molecular machinery in the marine vibrio strain for hydrogen production. An appropriate amount of metals such as Fe, Ni, Mo and Se has shown to affect the maturity and activities of the complex. Finally, in Chapter 4, a L_{27} Taguchi's orthogonal array design was used to study the effects of six metal elements; Fe, Ni, Mo, Se, Mg and Zn on H_2 productivity of *V. tritonius* AM2. The hybrid Taguchi method shows that Se has the highest influence on hydrogen productivity, followed by Mo > Ni > Zn > Mg > Fe. The method predicted the optimum formulation of metals would be at level 3, 1, 1, 1, 3 and 1 corresponding to 228 mg/L Fe, 0.05 mg/L Ni, 0.04 mg/L Mo, 0.05 mg/L Se, 24 mg/L Mg and 0.05 mg/L Zn. Eventually, a validation experiment of optimum formulation against the un-supplemented media has ruled out the needs of metals for efficient hydrogen production by this bacterium.

DOE with kinetic analyses allow better explanation on the fermentative process of AM2. OFAT design showed that AM2 is able to maintain hydrogen production at a broader range of NaCl concentration. The H_2 productivity starts to decrease at 16 g/L NaCl due to the decrease of substrate consumption. The regression models of FCC with RSM have helped to give a better understanding of the relationship among the 3 factors studied and their effects to responses, in particular, 100% SCE at a lower substrate supply could be of great merit for further development of continuous culture system using powdered macroalgae as a feedstock. Powdered macroalgae containing many metals and simultaneous study of all metals affecting hydrogen production via FHL pathway would involve many experimental runs. The Taguchi method has reduced the number of runs and identifying the metal and its level that could be the limiting factor for H_2 production. No significant hydrogen productivity in media between with and without metals could have an advantage in industry to set-up cost-effective media formulation. The hybrid Taguchi method also shows the needs for minor re-consideration and changes for better optimization. Many other factors could also affect the fermentative H_2 production. For further optimization, I proposed a preliminary screening using simple factorial design or Taguchi methods, followed with a Central composite or Box-Behnken design of RSM to find the true optimum of factors involved. Kinetic models can then be applied to understand the pattern of changes occurring in this system.