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TITTLE OF DISSERTATION

BIOCONVERSION OF AGAROSE IN RED SEAWEED BY ENGINEERED MICROBIAL CELLS FOR THE PRODUCTION OF BIOETHANOL

THE OUTLINE OF DISSERTATION

CHAPTER 1: INTRODUCTION

This chapter presents a statement of the global concern for the search of an alternative, sustainable and cleaner-burning energy to replace the dependent on petroleum based-fuel. Red seaweed was introduced as promising biomass for the production of bioethanol with a brief overview of current methods for agarose saccharification. The problems and challenges in current proposed method for agarose saccharification were stated together with the goal and specific objectives of the dissertation.

CHAPTER 2: LITERATURE REVIEW

This chapter reviews the literature on the potential of bioethanol as an alternative fuel. The drawbacks of first generation and second generation of bioethanol were previewed. Subsequently, the properties of red seaweed as promising biomass to overcome limitation from previous generation of bioethanol were stated together with current progress in agarose saccharification method for bioethanol production.

CHAPTER 3: BIOCONVERSION OF NEOAGAROBIOSE TO BIOETHANOL BY USING RECOMBINANT YEAST IN ETHANOL PRODUCTION FROM AGAROSE

The nucleotide sequence of *agaNash* gene that encoded for α-neoagarooligosaccharide hydrolase was determined. The aim of this chapter is to construct a recombinant *Saccharomyces cerevisiae* that can secrete α-neoagarooligosaccharide hydrolase, to hydrolyze neoagarobiose, thus release monosugar that include fermentable galactose and non-fermentable 3,6-anhydro-L-galactose. The recombinant yeast then convert the galactose to bioethanol.

CHAPTER 4: EFFICIENT PRODUCTION OF BIOETHANOL FROM AGAROSE BY USING RECOMBINANT BREVIBACILLUS AND YEAST

The focus of this chapter is to construct a recombinant *Brevibacillus choshinensis* that can secrete β-agarase enzyme. The β-agarase gene known as *agaA* derived from *Cellvibrio* sp. OA-2007 was cloned in expression vector known as pNY326. Efficient bioethanol production from agarose was achieved by simultaneous saccharification and fermentation of agarose by co-culture of recombinant β-agarase secreting *Brevibacillus* and recombinant *S. cerevisiae* secreting AgaNash.

CHAPTER 5: COMPLETE BIOCONVERSION OF OLIGOSACCHARIDE TO NEOAGAROBIOSE FOR HIGHER BIOETHANOL PRODUCTION

The main focus of this chapter is to increase bioethanol production from agarose. A novel β-agarase gene known as *agaMY* gene was amplify from genomic DNA of *Cellvibrio* sp. OA-2007 and expressed in *Escherichia coli*. The gene encoded for agarase AgaMY, was confirmed to have the ability to hydrolyze neoagarotetraose and neoagarohexaose and released neoagarobiose. Complete bioconversion of neoagarooligosaccharides to neoagarobiose was achieved. By introducing recombinant *S. cerevisiae* secreting AgaNash, higher concentration of ethanol was obtained.

CHAPTER 6: GENERAL CONCLUSIONS

With the results obtained from chapter 3, 4, and 5, the general conclusions associated with the bioconversion of agarose in red seaweed were presented together with the recommendations for further study in this chapter.