UNIVERSITI PUTRA MALAYSIA

PHYSICAL-CHEMICAL-ENZYMATIC PRETREATMENT PROCESS FOR BIOCONVERSION OF KITCHEN WASTE INTO FERMENTABLE SUGAR AS A FEEDSTOCK FOR BIOETHANOL PRODUCTION

HALIMATUN SAADIAH HAFID

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By

HALIMATUN SAADIAH HAFID

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the requirement for the Degree of Doctor of Philosophy

April 2017

Dedicated to my parents and my siblings

For their endless love, support and encouragement

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

PHYSICAL-CHEMICAL-ENZYMATIC PRETREATMENT PROCESS FOR BIOCONVERSION OF KITCHEN WASTE INTO FERMENTABLE SUGAR AS A FEEDSTOCK FOR BIOETHANOL PRODUCTION

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HALIMATUN SAADIAH HAFID

April 2017

Chairman : Nor 'Aini Abdul Rahman, PhD
Faculty : Biotechnology and Biomolecular Sciences

Sustainable conversion of municipal solid waste (MSW) into bioethanol taking into account the availability of the organic fraction of the waste which is kitchen waste that contains high carbohydrate, soluble sugar, starch, protein, lipid and other materials. It can be converted to fermentable sugar via hydrolysis and saccharification process which is still recognized as a rate-limiting step of the conversion process of kitchen waste to bioethanol. Hence, this study aimed to evaluate the physical, chemical, enzymatic and combination of pretreatment at enhancing the hydrolysis and maximizing the yield of fermentable sugar produced. This study is divided into three parts of pretreatment by (1) Hydrothermal and dilute acid; 2) Enzymatic pretreatment; 3) Combination of physical-chemical and enzymatic pretreatment, and followed by 4) Production of bioethanol using saccharified kitchen waste using locally isolated yeasts; *Saccharomyces cerevisiae*, *Candida parasilosis*, and *Lachancea fermentati*.

Initially, hydrolysis of kitchen waste by hydrothermal and dilute acid systems were carried out by varying the reaction temperatures (80, 90, 100°C) hydrolysis reactant; hot distilled water (hydrothermal), hydrochloric acid (HCl) and sulphuric acid (H_2SO_4) at different concentrations (0.5 - 2.0%) and evaluated using kinetic modelling approach by gPROMS software. A significant improvement of fermentable sugar production by 40.6% was observed at optimize condition of 1.5% HCl and 44.9% using 1.0% H_2SO_4 at 90°C as compared to hydrothermal pretreatment. The hydrolysis rate coefficient at 90°C for fermentable sugars production, $k_{r=1}$, was 0.68 $gL^{-1}min^{-1}$ for hydrothermal system and 1.61 and 1.88 $gL^{-1}min^{-1}$ in HCl and H_2SO_4 catalysed system, respectively. Enhanced hydrolysis of kitchen waste for fermentable sugar production was achieved by hydrothermal and dilute acid pretreatment.

Meanwhile, response surface methodology (RSM) technique is adopted in enzymatic pretreatment for a prediction of optimal condition of independent variables (pH, temperature, glucoamylase activity, kitchen waste loading and hydrolysis time) on fermentable sugar production and degree of saccharification. Quadratic RSM predicted maximum fermentable sugar production of 62.79 g/L and degree of saccharification (59.90%) at the optimal conditions; pH 5, temperature 60°C, glucoamylase activity of 85 U/mL and utilized 70 g/L of kitchen waste as a substrate at 10 hours hydrolysis time. The verification experiments successfully produced 65.71 ± 0.7 g/L of fermentable sugar with 55.3 ± 0.4% degree of saccharification which 31.4% higher than non-optimized condition indicating that the developed model was successfully used to predict fermentable sugar production at more than 90% accuracy.

The experiment was continued by applying single and combination pretreatments by hydrothermal, mild acid pretreatment of HCl and H_2SO_4 and with enzymatic hydrolysis by glucoamylase. The maximum total fermentable sugar produced after combination pretreatment by 1.5% HCl and glucoamylase produced 94.45 g/L of fermentable sugar consisted of 93.25 g/L glucose, 0.542 g/L sucrose, 0.348 g/L maltose, and 0.321 g/L fructose. An increase of 55.8% and 91.8% of fermentable sugar production was obtained by comparing with single glucoamylase and 1.5% HCl pretreatment, respectively. From FTIR analysis, the decrease of aliphatic absorbance bands of polysaccharides at 2851 and 2923 cm^{-1} and the increase on structures of carbonyl absorbance bands at 1600 cm^{-1} reflects the progress of the kitchen waste hydrolysis to fermentable sugars. For total cost and profit estimation, combination of 1.5% HCl and glucoamylase pretreatment was the most profitable process as the minimum selling price of glucose was USD 0.101/g kitchen waste. The combination pretreatment method was successfully enhance the production of fermentable sugar from kitchen waste.

Production of fermentable sugar using acid-pretreated and enzymatic hydrolysis of kitchen waste was then conducted in 2L of bioreactor. The results suggested that a significant increase in fermentable sugar production to 103.4 ± 0.04 g/L (2.04-folds) with conversion efficiency of 86.8% was observed via sequential acid-enzyme pretreatment as compared to dilute acid (42.4%) and glucoamylase enzyme (50.6%), respectively. An increased in total fermentable sugar to 150.5 ± 0.11 g/L which consist of glucose (128.47 g/L), fructose (6.24 g/L), sucrose (5.59 g/L) and maltose (10.18 g/L) was successfully recovered after downstream processing. The fermentable sugars obtained were subsequently converted to bioethanol by locally isolated yeasts; *Saccharomyces cerevisiae*, *Candida parasilosis*, and *Lachancea fermentati* produce ethanol yield ranging from 0.45 g/g to 0.5 g/g after 24 h which was equivalent to 82.06 - 98.19% of conversion efficiency based on theoretical yield that was comparable with using commercial glucose. The finding indicates that kitchen waste can be considered as a promising substrate for bioethanol production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PROSES PRA-RAWATAN SECARA FIZIKAL-KIMIA-ENZIM BAGI PENGHASILAN GULA TERFERMENTASI DARIPADA SISA BUANGAN DAPUR SEBAGAI BAHAN MENTAH PENGHASILAN BIOETANOL

Oleh

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Penukaran lestari sisa buangan pepejal bandaran (MSW) kepada bioetanol mengambil kira kandungan organik iaitu sisa buangan dapur yang mengandungi karbohidrat yang tinggi, gula terlarut, kanji, protin, lipid dan bahan yang lain. Ia boleh ditukarkan kepada gula fermentasi melalui proses hidrolisis dan pensakaridaan yang masih menjadi faktor-pengehad kepada proses penukaran sisa buangan dapur kepada bioetanol. Oleh itu, kajian ini menilai pelbagai kaedah pra-rawatan fizikal, kimia, enzim dan kombinasi pra-rawatan untuk meningkatkan hidrolisis dan memaksimakan penghasilan gula. Kajian ini terbahagi kepada tiga bahagian pra-rawatan melalui (1) Hidroterma dan pemangkin asid-cair; (2) Pra-rawatan enzim; (3) Kombinasi pra-rawatan fizikal, kimia dan enzim; dan diikuti oleh 4) Penghasilan bioetanol menggunakan hidrolisit pensakaridaan sisa buangan dapur oleh yis yang telah dipencilkan dari kawasan tempatan; *Saccharomyces cerevisiae*, *Candida parasilosis,* dan *Lachancea fermentati*.

Permulaannya, hidrolisis sisa buangan dapur melalui kaedah sistem hidroterma dan asid cair dijalankan dengan mempelbagaikan suhu tindakbalas (80, 90, 100°C), bahan tindak balas; air suling panas (hidroterma), asid hidroklorik (HCl) dan asid sulfurik (H_2SO_4) pada kepekatan berbeza (0.5-2.0%) dan dinilai menggunakan model kinetik oleh perisian gPROMS. Peningkatan ketara gula terfermentasi sebanyak 40.6% diperoleh pada keadaan optimum 1.5% HCl dan 44.9% pada keadaan 1.0% H_2SO_4 berbanding pra-rawatan hidroterma. Pekali kadar hidrolisis pada 90°C penghasilan gula terfermentasi ialah $k_{r=1}$, 0.68 $gL^{-1}min^{-1}$ bagi sistem hidroterma manakala 1.61 dan 1.88 $gL^{-1}min^{-1}$ bagi sistem HCl dan H_2SO_4. Peningkatan gula terfermentasi dalam hidrolisis sisa buangan dapur melalui pra-rawatan hidroterma dan asid cair berjaya dicapai.

Sementara itu, kaedah permukaan tindakbalas (RSM) digunakan semasa pra-rawatan enzim untuk menganggar keadaan optimum oleh faktor-faktor bebas (pH, suhu, aktiviti glukoamilase, beban sisa buangan dapur, dan masa hidrolisis) kepada penghasilan gula fermentasi dan tahap pensakaridaan. Kuadratik RSM meramalkan penghasilan gula terfermentasi tertinggi ialah 62.79 g/L dan tahap pensakaridaan (59.90%) pada keadaan optimum; pH 5, suhu 60°C, aktiviti glukoamilase 85 U/mL dan menggunakan 70 g/L sisa buangan dapur sepanjang 10 jam masa hidrolisis. Eksperimen pengesahan berjaya menghasilkan 65.71 ± 0.7 g/L gula terfermentasi dengan 55.3 ± 0.4 % tahap pensakaridaan dimana 31.4% lebih tinggi berbanding tanpa keadaan optima menunjukkan model yang direka bentuk berjaya digunakan untuk meramal penghasilan gula fermentasi dengan lebih 90% ketepatan.

Eksperimen diteruskan dengan pra-rawatan tunggal dan kombinasi pra-rawatan oleh hidroterma, pra-rawatan asid cair oleh HCl dan H_2SO_4 dan hidrolisis enzim oleh glukoamilase. Jumlah penghasilan gula terfermentasi tertinggi diperolehi melalui kombinasi pra-rawatan oleh 1.5% HCl dan glukoamilase menghasilkan 94.45 g/L gula terfermentasi dengan mengandungi 93.25 g/L glukosa, 0.542 g/L sukrosa, 0.248 g/L maltose dan 0.321 g/L fruktosa. Peningkatan sebanyak 55.8% dan 91.8% gula terfermentasi diperolehi berbanding pra-rawatan tunggal menggunakan glucoamylase dan 1.5% HCl sahaja. Dari analisa FTIR, pengurangan kuantiti penyerapan kumpulan alifatik polisakarida pada 2851 dan 2923 cm^{-1} dan peningkatan kuantiti penyerapan pada struktur karbonil di 1600cm^{-1} menggambarkan tindakbalas hidrolisis sisa buangan dapur kepada gula terfermentasi. Berdasarkan analisa anggaran kos dan untung, kombinasi 1.5% HCl dan glukoamilase adalah pra-rawatan yang menguntungkan dengan memberikan harga jualan minimum glukosa sebanyak USD 0.101/g sisa buangan dapur. Teknik kombinasi pra-rawatan berjaya meningkatkan penghasilan gula terfermentasi daripada sisa buangan dapur.

Penghasilan gula terfermentasi dari sisa buangan dapur terawat asid dan hidrolisis enzim kemudiannya dilakukan di dalam bioreactor berkapasiti 2 L. Keputusan menunjukkan peningkatan ketara penghasilan gula terfermentasi kepada 103.4 ± 0.04 g/L (2.04-kali ganda) dengan kecekapan penukaran 86.8% diperolehi melalui jujukan pra-rawatan asid-enzim berbading pra-rawatan asid cair (42.4%) dan pra-rawatan enzim glukoamilase (50.6%). Peningkatan gula fermentasi kepada 150.5 ± 0.11 g/L mengandungi glokosa (128.47 g/L), fruktosa (6.24 g/L), sukrosa (5.59 g/L) dan maltose (10.18 g/L) berjaya diperolehi selepas proses hiliran dilakukan. Gula terfermentasi kemudiannya ditukarkan kepada bioetanol oleh yis yang dipencilkan; *Saccharomyces cerevisiae*, *Candida parasilosis,* dan *Lachancea fermentati* menghasilkan etanol tertinggi diantara 0.45 g/g - 0.5 g/g selepas 24 jam penapaian bersamaan dengan 82.06 - 98.19% kecekapan penukaran berdasarkan hasil teori dan setanding dengan glukosa komersil. Penemuan ini menunjukkan sisa buangan dapur dianggap substrat yang berpotensi untuk penghasilan bioetanol.

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I certify that a Thesis Examination Committee has met on 3 April 2017 to conduct the final examination of Halimatun Saadiah Hafid on her thesis entitled "Physical-Chemical-Enzymatic Pretreatment Process for Bioconversion of Kitchen Waste into Fermentable Sugar as Feedstock for Bioethanol Production" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BOD	Biological Oxygen Demand
CCD	Central composite design
CHO	Carbohydrate
COD	Chemical oxygen demand
H_2SO_4	Sulfuric acid
HAc	Acetic acid
HCl	Hydrochloric acid
HLa	Lactic acid
HPLC	High Performance Liquid Chromatography
HPr	Propionic acid
KH_2PO_4-	Potassium dihydrogen phosphate
MESP	Minimum bioethanol selling price
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
MSW	Municipal Solid Waste
NaOH	Sodium hydroxide
RSM	Response Surface Methodology
TKN	Total Kjeldahl Nitrogen
TS	Total Solid
TSS	Total Suspended Solid
YPD	Yeast Peptone Dextrose

CHAPTER 1

INTRODUCTION

1.1 Research Background

The management of municipal solid waste (MSW) has been continuously be a major challenge in Malaysia attributed to the rapid urbanization and industrialization especially in urban areas. Along with the economic growth, Malaysian population has tremendously increased at the rate of 2.4% per annum, thus increase the consumption rate and accelerate the daily generation of solid wastes from 0.5-0.8 kg/person/day to 1.7 kg/person/day in major cities (Budhiarta et al., 2012). A recent study reported that Malaysians produced 33,000 tons of solid waste daily in 2012, exceeding the government's projected waste production of 30,000 tonnes daily by 2020 (Mokhtar, 2013). A typical MSW management system applied landfill as a primary choice of handling these waste, however, it displays an array of problem of crude open dumping, water pollution from the leachate generation and the breeding of flies and vermin that becoming the major environmental and public health problem due to the high concentration of organic material (71.6%) and moisture content present (Latifah et al., 2009).

The organic material of MSW is mainly referring to food or kitchen waste that is usually discharged from restaurants, kitchens, and cafeterias and leftovers from food industry. Kitchen waste is highly biodegradable comprises a rich carbon and nutrient such as carbohydrate, soluble sugar, starch, protein, lipids and other inorganic materials (Moon and Song, 2011). Pleissner and Lin (2013) reported the composition of kitchen waste consisting of approximately 60% carbohydrate, 20% proteins, 10% lipids and 10% of other materials. Current research performed is utilizing kitchen waste an alternative nutrient medium for conversion to organic acids (Omar et al., 2009; Bo et al., 2007), biogas (Ma et al., 2011; Munda et al., 2012), and bioethanol (Uncu and Cekmecelioglu, 2011; Tang et al., 2008). Various application of kitchen waste makes it a valuable raw material for the recovery of nutrient needed, however, its hydrolysis of particulates solid is still rate-limiting step in its application in many biotechnological process (Pleissner and Lin, 2013). The differences in individual biodegradability of kitchen waste compositions, such as carbohydrate, starch, protein, fat and fiber generates various degradation characteristics and may cause decreases in hydrolysis efficiency (Moon and Song, 2011; Moon et al., 2009). Hydrolysis of carbohydrate in kitchen waste may result in the breakdown of glycosidic bonds with releasing polysaccharides as monosaccharides, which are more amenable to fermentation especially for bioconversion to bioethanol (Kiran et al., 2014). Hence, the hydrolysis of kitchen waste can be performed by physical that normally involved thermal hydrolysis, chemical by acid hydrolysis, and biological by enzymatic hydrolysis and combinations of them.

Generally, thermal hydrolysis may lead to partial degradation of sugars and other nutritional components due to Maillard reaction that make the amounts of targeted sugar and amino acids decreased (Kiran et al., 2014), whereas, chemical hydrolysis by acid may penetrate and disrupt the structure of biomass more easily thus increase the hydrolysis rate, however, the rapid degradation of sugars particularly glucose and generation of unnecessary compound make it less advantages (Li et al., 2012). In contrast, enzymatic hydrolysis by α-amylase and glucoamylase is more environmentally friendly for obtaining sugars; but, the low enzymatic accessibility due to the nature and recalcitrant of the biomass is a key problem for the kitchen waste-to-bioethanol process (Kumar et al., 2009). Moreover, most of the enzymatic processes are too slow thus limiting application at the industrial level and the excessive cost of enzymes may increase the overall processing cost (Duvernay et al., 2013; Vavouraki et al., 2012). In search of the viable alternative substrate for fermentable sugar and bioethanol production, the feasibility of the kitchen waste need to be further explored as its contain high nutritional value and organic content with a large volume of waste generated apart of its underused resources. As a pretreatment remains the most hurdles in producing technical and economically viable fermentable sugar and bioethanol production from biomass, the integrated pretreatment using a combination methods of physical and chemical by dilute acid solution together with the enzymatic saccharification is a potential efforts directed towards increasing the biodegradability of complex biomass particularly organic kitchen waste to monomeric sugars (Fred-Guelfo et al., 2011).

The integrated pretreatment has been pursued as a promising approach to break the structure of kitchen waste and expose the starch component and cellulose to enzyme action for higher efficiency of sugars production, decrease formation of inhibitory and simultaneously shortening the process time (Mood et al., 2013). The interest in utilizing integrated pretreatment is facilitate by the need to improve the efficacy of kitchen waste degradation, with a more recent focus on operating at high concentration of solid matters for fermentable sugar production. Modenbach and Nokes, (2013) reported at high solid content of biomass has been identified as the largest contributor in achieving high yields sugar in a timely manner of the kitchen waste to bioethanol conversion process, mainly because a significant portion of sugars produced translate into higher bioethanol concentration. In a study conducted by Tang et al. (2008), the combination of mechanical treatment by compact chopper and glucoamylase produced 74.1 g/L of glucose whereas about 200 g/L of glucose was successfully recovered using the same combination pretreatment (Yan et al., 2010). Vavouraki et al. (2014) reported an increase of ~300% of glucose concentrations after subjected to pretreatment with acid or alkaline in combination with enzymatic hydrolysis. Besides, combination thermo-chemical pretreatment improve the digestibility of solid content for high rate of hydrolysis (Li et al., 2015).

In developing a technical and economically feasible pretreatment process, a lot of parameters such as type of pretreatment (physical, chemical, enzymatic), operating temperature, pH, pretreatment time, kitchen waste concentration and other unit operations involved need to be optimized to ensure higher production of fermentable sugar obtained with lower production of inhibitors and lesser environmental impacts. Bioprocess modelling and optimization has traditionally been carried out using One Variable at a Time (OVAT) and Response Surface Methodology (RSM) to evaluate the process parameters to the output products. RSM is favored by many researchers due to rapid and reliable statistical analysis and generate interactive effects of input process parameters of the complex substrate such as kitchen waste (Hafid et al., 2011). An extensive understanding of the integrated pretreatment process on the kitchen waste constituent, physical and chemical changes involved, possible generation of toxic compound is needed to evaluate the overall performance and efficiency of selected pretreatment methods. Apart from the technical aspect of fermentable sugar and bioethanol production, the effect of the pretreatment process and parameter on the economic analysis of the fermentable sugar is reflected in the cost estimation analysis. The excessive cost of pretreatments may render the overall process and economically unsustainable. In addition, the chosen pretreatment must be attractive to be applied in which it could save time, chemical used, laboratory apparatus and manpower. The most appropriate pretreatment methods takes into account the technical and economic evaluation towards the sustainable decision making process.

This study present a unique set of understanding on bioconversion of kitchen waste into fermentable sugars by integrated physical, chemical and enzymatic pretreatment. The bioethanol production was performed utilizing the fermentable sugar obtained in the kitchen waste sacharified liquid by three locally isolated yeasts; a single culture of *Saccharomyces cerevisiae*, *Lachancea fermentati* and *Candida parasilosis* in separate hydrolysis and fermentation process. The feasibility of converting kitchen waste-fermentable sugars into bioethanol is evaluated and expected to increase interest in utilizing kitchen waste as a feedstock for potential development of bioethanol production.

1.2 Problem Statements

In literature, very limited studies focus on the utilization of kitchen waste for fermentable sugar and bioethanol production as compared to lignocellulosic biomass probably due to the heterogeneity and inconsistent compositions and laborious separation process of kitchen waste. An establishment of a standard preparation of kitchen waste need to be develop to overcome varies composition of kitchen waste before being subjected to any pretreatment for valuable products. In addition, very rare studies reported on the effect of integrated and combination pretreatment of physical and chemical prior to enzymatic hydrolysis as compared to single pretreatment in which is usually claimed to be much simpler with low-cost technology. Pretreatment strategies should be establish on a basis of the heterogeneity of the biomass to avoid selective deconstruction and fractional conversion to other by-product. A major demerit of single pretreatment is apportion of multiple products thereby reduce the concentration of main fermentable sugars. Furthermore, an appropriate pretreatment is crucial in dealing with high solid content of the biomass, particularly kitchen waste to ensure complete degradation occurs for higher yield of fermentable sugar. Despite of the many pretreatment method tested in the literature, there is still a need to establish more defined pretreatment based approach for kitchen waste conversion and evaluate the processing conditions for an effective fermentable sugar production.

1.3 Objectives

This study investigated the improvement of kitchen waste hydrolysis by single and combination pretreatments method through physical, chemical, and enzymatic approaches for higher yield of fermentable sugar production with a maximum degradation efficiency of the kitchen waste. Upon completion of the pretreatment, the hydrolysate was recovered and inoculated with ethanol-fermenting microorganisms and subjected to bioethanol production in separate hydrolysis and fermentation (SHF) process. Therefore, the specific objectives of this study were:

1) To investigate the environmental factors of kitchen waste hydrolysis for fermentable sugar production by hydrothermal and dilute acid pretreatment methods.
2) To optimize the enzymatic pretreatment method of kitchen waste for fermentable sugar production using response surface methodology.
3) To enhance fermentable sugar production from kitchen waste hydrolysis using combination of physical-chemical-enzymatic pretreatment method.
4) To produce fermentable sugar using enzymatic sequential batch of kitchen waste treated acid in 2L bioreactor for bioethanol production by locally isolated yeasts.

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