APPLICATION OF WATER HYACINTH IN WASTEWATER TREATMENT AND CELLULOSIC ETHANOL PRODUCTION

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To

This thesis is dedicated to my family

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ABSTRACT

Water pollution and depletion of fossil fuels are crucial issues which significantly affect sustainability of water resources and usage of non-renewable energies. Phytoremediation technique is an effective method to treat different types of wastewater using plants in an economical way. Lignocellulosic biomass as a renewable resource can be replaced with fossil fuels. In this research, the lignocellulosic aquatic plant, water hyacinth (WH) was propagated in fabricated tanks to evaluate wastewater purification and ethanol production efficiency. Wastewater treatment was performed in two modes (batch and continuous) and the results were compared with the Interim National Water Quality Standards, Malaysia River classification (INWQS) and Water Quality Index (WQI). The matured plants were harvested at maximum growth rate with the highest sugar content. Then, the WH was dried, and four pretreatments, namely ionic liquid, acidic, alkali and microwave-alkali were performed to obtain optimum carbohydrates content. Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) were used for compositional and structural analysis on WH before and after pretreatments. Simultaneous Saccharification and Fermentation (SSF) was performed to obtain a high level of sugars which could be converted into ethanol. In the fermentation process, the inoculation of yeast (*Saccharomyces cerevisiae*) and commercial cellulase enzyme from (*Aspergillus niger)* were used. Total reducing sugar and ethanol concentration were measured by Dinitrosalicylic Acid (DNS) method and gas chromatography. In the batch system, some parameters such as pH, chemical oxygen demand (COD), phosphate (PO_3^{-4}), nitrate (NO_3^-), ammoniacal nitrogen (NH_3) and total organic carbon (TOC) were reduced and the water quality has improved from class III to II. In the continuous system, the removal efficiency 38% to 96% of ten parameters was obtained. The highest ethanol yield was produced by alkali-treated WH with 6.2 ± 0.4 g/L with 0.12 g/g WH/ethanol in 48 hours followed by microwave-alkali, acid and ionic liquid pretreatments.

ABSTRAK

Pencemaran air dan pengurangan bahan api fosil adalah isu-isu penting yang ketara dan memberi kesan kepada kelestarian sumber air dan penggunaan tenaga yang tidak boleh diperbaharui. Teknik Fitopemulihan merupakan kaedah yang berkesan untuk merawat pelbagai jenis tumbuhan air sisa dengan menggunakan cara yang menjimatkan. Lignoselulosa tumbuhan sebagai sumber yang boleh diperbaharui dan boleh diganti dengan bahan api fosil. Dalam kajian ini, akuatik lignoselulosa tumbuhan, iaitu keladi bunting (WH) telah ditanam di dalam tangki yang direka khas untuk menilai pembersihan air sisa dan kecekapan pengeluaran etanol. Rawatan air sisa telah dilaksanakan dalam dua kaedah (kelompok dan berterusan) dan keputusan yang diperolah akan dibandingkan dengan Piawaian Interim Kualiti Air Kebangsaan, klasifikasi River Malaysia (INWQS) dan Indeks Kualiti Air (WQI). Tumbuh-tumbuhan yang matang dituai pada kadar pertumbuhan maksimum dengan kandungan gula yang tinggi. Kemudian, WH yang telah kering bersama empat pra-rawatan iaitu cecair ionik, asid, alkali dan ketuhar gelombang mikro-alkali telah dilaksanakan untuk mendapat kandungan karbohidrat yang optimum. Scanning Electron Microscopy (SEM) dan Fourier Transform Infrared (FTIR) telah digunakan untuk analisis komposisi dan struktur ke atas WH sebelum dan selepas pra-rawatan. Saccharification serentak dan Penapaian (SSF) telah dilaksanakan untuk mendapat tahap gula yang tinggi yang boleh ditukar menjadi etanol. Dalam proses penapaian, inokulasi yis (Saccharomyces cerevisiae) dan enzim cellulase komersial dari (Aspergillus niger) telah digunakan. Jumlah pengurangan gula dan kepekatan etanol diukur oleh kaedah Acid Dinitrosalicylic (DNS) dan kromatografi gas. Dalam sistem kumpulan, beberapa parameter seperti pH, keperluan oksigen kimia (COD), fosfat (PO_3^{-4}), nitrat (NO_{3-}), nitrogen ammonia (NH_3) dan jumlah karbon organik (TOC) telah dikurangkan dan kualiti air meningkat daripada kelas III ke II. Dalam sistem yang berterusan, kecekapan penyingkiran 38% kepada 96% daripada sepuluh parameter telah diperolehi. Hasil keputusan menunjukkan bahawa kadar tinggi bagi penghasilan etanol adalah berhubung kait dengan; penggunaan WH dengan 6.2 ± 0.4 g / L dengan 0.12 g / g WH / etanol dalam 48 jam diikuti dengan ketuhar gelombang mikro-alkali, asid dan pra-rawatan cecair ionik.

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LIST OF ABBREVIATIONS

BGL	-	β-glucosidase
BOD	-	Biochemical Oxygen Demand
COD	-	Chemical Oxygen Demand
d	-	Day
DBP	-	Desa Bakti pond
DO	-	Dissolved oxygen
DOE	-	Department of Environment
EC	-	Electricity Conductivity
GC	-	Gas Chromatography
gds	-	gram dry substrate
h	-	Hour
IL	-	Ionic Liquid
INWQS	-	Interim National Water Quality Standards
NB	-	Nutrient Broth
nm	-	Nanometer (10^{-9} m)
OD	-	Optical Density
PDA	-	Potato Dextrose Agar
RGR	-	Relative growth rate
SHF	-	Separate Hydrolysis and Fermentation
SSF	-	Simultaneous Saccharification and Fermentation
TDS	-	Total Dissolved Solids

TOC	-	Total Organic Carbon
TRS	-	Total Reducing Sugar
TSS	-	Total Suspended Solids
WH	-	Water Hyacinth
WQI	-	Water Quality Index

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Water hyacinth (*Eichhornia crassipes)* has drawn worldwide attention being a harmful weed. It is also considered as a native of South America, invasive and free-floating aquatic macrophyte. Moreover, the rapid growth and quick spreading of Water Hyacinth (WH) cause serious problems for navigation and irrigation (Gopal, 1987). Water hyacinth growth highly depends on the seasonal changes and availability of nutrients in water. For example, the best season for effective cultivation is in summer although it can grow in winter. It competes with almost all the other species growing in their vicinity, which decreases the biodiversity (Craft *et al.,* 2003). It devastates aquatic environment and costs billions of dollars every year in control. As a result, many environmentalists are in favor of the removal of WH from fresh water ecosystem. They discovered that there are several ways to remove this problematic weed from water such as using biological and mechanical methods. However, after removal, it can be used in many useful and eco-friendly processes to keep our environment clean (Gupta *et al.,* 2012).

Water pollution is a significant environmental issue that has been increasing exponentially with urbanization and modernization along with the population growth of the world. As discussed by Ling, (2010), water pollution is a crucial issue in Malaysia which significantly affects the sustainability of water resources and management. Phytoremediation technique is an effective method to treat different types of wastewater using plants in an economical way. Important mechanisms for phytoremediation technique are uptake, accumulation, translocation and metabolism of micro-contaminants (Lv *et al.,* 2016). In this technique, floating aquatic plants such as WH can be used to uptake organic and inorganic matter from wastewater. The abilities of WH such as higher growth rate than other aquatic plants, high absorption efficiency and low operation cost imply that using this plant can be considered as a suitable green plant for the treatment of wastewater (Gupta *et al.,* 2012).

At present, depletion of fossil fuels in near future is predictable by continuous over-utilization of natural energy sources (Jambo *et al.,* 2016). Additionally, lack of energy resources is in an alarming stage due to globalization and increasing demand for energy. In this situation, the researchers tried to find alternative sources of energy that are not related to fossil oil and natural gas (Bradshaw, 2010). On the other hand, the concern on greenhouse effect is an important reason for interest in renewable energy sources. For example, ethanol due to its potential as an alternative automotive fuel has attracted worldwide attention. In addition, concerns towards CO_2 emissions and associated climate change have instigated an accelerated research and production of renewable energy resources (Sanchez and Cardona, 2008). Therefore, these issues have driven many countries to become interested in the use of biofuels as a replacement source of renewable and cheap energy (Tye *et al.,* 2016). Water hyacinth is a type of lignocellulosic material that is potentially available in the tropical region of the world. (Ganguly *et al.,* 2012; Sing and Bishnoi, 2013).

1.2 Background of the study

For a phytoremediation system to work efficiently, an optimal plant growth is the key parameter. The growth rate of WH is found to be dependent on the concentration of dissolved nitrogen (N) and phosphorus (P) present in the wastewater. In addition, the propagation of WH depends on the availability of light, nutrients and the abundance and stability of weevil populations (Williams *et al.,* 2005). The most common aquatic macrophytes among the floating-leaved plants being employed in wastewater treatment are WH and water lettuce (John *et al.,* 2008, Maine *et al.,* 2004; Mishra *et al.,* 2008). There are many studies on the removal of a wide range of pollutants from wastewater by WH (Patel, 2012; Gupta *et al.,* 2012). Significant removal of inorganic nitrogen (nitrate), ammoniacal nitrogen (NH_3-N), total N, phosphorus and total P have been reported by using WH in nutrient rich wastewater (Lu, 2009).

Agricultural products like corn, wheat and sugarcane are defined as the first generation, lignocellulosic biomass as the second generation and algal biomass as the third generation of raw materials for bioethanol production (Baeyens *et al.,* 2015). There are several advantages in the production of ethanol in comparison with gasoline, namely the utilization of an abundant and cheap source of renewable resources, reduction in greenhouse gas emission and toxic substances by carbon neutrality and the significant benefits for rural community and social aspect of sustainability (Lin and Tanaka, 2006). A world production of 50 million m^3 in 2007 and in excess of 100 million m^3 in 2012 of bio-ethanol was reported as an alternative renewable fuel with the largest potential to replace fossil-derived fuels. Brazil and the United States represent approximately 80% of the world supply of bioethanol (Renewable Fuels Association, 2013). This source of energy also has a potential for a significant reduction of greenhouse gas emissions of fossil fuel origin as well by carbon sequestration (Dias *et al.,* 2013).

Lignocellulosic biomass consists of carbohydrates that can be derived from monosaccharide and disaccharide. On the other side, carbohydrates are comprised of cellulose and hemicellulose that are tightly wrapped by lignin. Water hyacinth, which contains large quantity of cellulose but small content of lignin, can be converted into bioethanol by enzymes. The conversion of biomass into hydrocarbons utilizes a complex but reasonably well understood biochemical route (Shafiei *et al.,* 2015). Large quantities of cellulase and hemicellulose can be produced by genetically engineered microorganisms (Dashtban *et al.,* 2010). Fermentable sugars can be obtained by these enzymes from lignocellulosic compounds like agricultural residues (e.g. corn stover, straw, sugar cane bagasse) and energy crops (e.g. switchgrass) (Mousdale 2008). Genetically modified fermentative and cellulolytic microorganisms are crucial to increase ethanol yield and productivity under stress conditions such as high temperature (Chen, 2009). Due to importance of hydrolytic pre-treatment for cellulose, hemi-cellulose and lignin separation, the conversion of lignocellulosic biomass is an expensive and difficult process. After separation of these materials, the hexose and pentose sugars can be fermented into different alcohols (Anca-Couce, 2016).

Increasing the yield of bioethanol and improvement of the process of simultaneous saccharification and fermentation can be obtained from a study on the decomposition of lignocellulosic biomass (Kotarska et al, 2015). Compositional analysis of lignocellulose before and after pretreatment, is used in almost all second-generation biofuel production studies. According to (Karimi and Taherzadeh, 2016a), an accurate analysis is essential to evaluate the biofuel production. The efficiency of pretreatment methods highly depends on a variety of analytical methods that address their image and composition which can be obtained from **S**canning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) analysis.

Using appropriate fermentation substrate and suitable process technology can increase the yield of ethanol production by microbial fermentation (Brooks, 2008). In order to increase the bioethanol yield, these pretreatment methods are applied to decrease the recalcitrance of lignocellulosic feedstock. Pretreatment can decrease crystallinity of cellulose, increase accessibility of polymers by enzymes and reduce lignin content and these functions depend on the mode of the pretreatment methods (Zheng *et al.,* 2014).

1.3 Problem statement

Water is the delicate part of the environment, which is essential for human life and industrial development. Water pollution is not a recent environmental issue and has become a hot topic with urbanization and modernization. Malaysia's major source of surface water contributes some 97% of the total water supply (Gasim et al, 2009). Phytoremediation technique can be applied to treat different types of wastewater that in comparison to other techniques is an environmentally friendly. In literature, WH showed a greater uptake ability in comparison to other aquatic plants like water lettuce (Ismael *et al.,* 2015), water morning glory (Loan *et al.,* 2014) and azolla (Anandha *et al.,* 2015). Water hyacinth has amazing ability to remove different types of pollutants such as heavy metals, organic and inorganic compounds from aquatic environments. It grows quickly even in extreme conditions and no land space is needed for its cultivation (Akinbile *et al.,* 2012; Akinbile *et al.,* 2016).

In many countries, fossil fuel usage is a big problem that is regarded as non-renewable energy. Malaysia currently relies on fossil fuels for over 90% of its power generation. With such a high demand of energy usage and abundant biomass resources can utilize the biomass residue (Hu, 2016). In contrast to food crops for biofuel production, the utilization of lignocellulosic biomass reduces the carbon footprint and greenhouse gas emission effects (Luque *et al.,* 2008; Govumoni *et al.,* 2013). In this regard, biodiesel and bio-ethanol are the most common biofuels and can replace fossil diesel and gasoline in car engines (Mata *et al.,* 2010).

Water hyacinth is an aquatic and abundant plant and a good candidate for production of substitute fuels such as bioethanol due to the high percentage of carbohydrates. Interestingly, WH has a low amount of lignin (10%), high amount of cellulose (25%) and hemicellulose (35%). The economic potential of the biofuel production can be improved by using low-lignin biomass in comparison with lignin-rich biomass (Bhatt and Shilpa, 2014). According to Singh and Bishnoi, (2012), three main operations are needed to convert lignocellulose to bioethanol; the pretreatment is necessary to liberate cellulose and hemicellulose before hydrolysis, the hydrolysis of cellulose and hemicellulose to produce fermentable sugars and fermentation of reducing sugars. In this way, the most common and traditional microorganism used in industrial bioethanol production is the yeast *Saccharomyces cerevisiae* (Dionisi *et al.,* 2015).

1.4 Objectives of the study

The objectives of this research are as follows:

1. To determine the maximum rate of organic and nutrient removal from domestic wastewater stabilization pond using local species of WH in batch and continuous system

2. To identify the potential parts of WH suitable to produce sugars in large quantities and the correlation to the growth rate of the biomass
3. To evaluate the efficiency of different pretreatment methods based on compositional and structural analyses in morphology of raw and treated WH by SEM and FTIR

4. To establish high rate production system of ethanol by fermentation by evaluating different pretreatment methods

1.5 Scope of the study

The scope of this research is to study the ability of WH to remove pollutants from domestic wastewater that leads to produce a high amount of bioethanol by simultaneous saccharification and alcohol fermentation. The efficiency of nutrient uptake by matured WH from domestic wastewater in fabricated tanks was compared in batch (no water flow) and continuous system (with water flow). Then, best part of WH in terms of high level of available sugars was selected based on preliminary study. Although, biomass should be harvested at the suitable stage of maturity that could produce highest reducing sugars with a suitable pretreatment.

Various pretreatment methods such as alkali, acidic, Microwave-alkali and Ionic liquid were applied to enhance the recovery ratio of sugars from the biomass. In addition, the effect of different pretreatments on composition of WH was investigated. Then, the conversion ability of glucose fermenting yeast (*Saccharomyces cerevisiae)* for conversion of sugars to ethanol was evaluated. Furthermore, by using cellulase the conversion of sugar to ethanol (yield) would be higher than absence of enzyme. Overall, WH can uptake nutrient from wastewater for its growth that leads to wastewater polishing. It is predicted that the best part in terms of higher sugar and carbohydrates content and lower lignin content would be more feasible for ethanol production.

1.6 Significance of the study

Effluent from oxidation pond with a wide range of pollutants is being released into rivers and has harmful impacts on human health. Studies have shown that WH was effective in removing wide range of nutrients from different types of wastewater. During this study, a free available macrophyte WH was applied as a multi-functional plant to remove contaminants from oxidation pond as well as ethanol production in presence of cellulase. The whole process is like a sequence as: the efficiency of fermentation is based on the amount of the sugars related to nutrient uptake from waste water and it is predicted that more sugar obtained can produce more ethanol. In this study, the conversion of waste to wealth can be considered as the most important issue.

This study is significant in regard to:

- Purification of domestic waste water by WH by uptake of organic and inorganic compounds
- Decrease the air pollution and CO_2 fixation by cultivation of this plant in the environment
- Using WH as a raw material which is abundant and free substrate for wide range of application as a multi-functional plant especially in tropical regions like Malaysia
- Ethanol produced from WH as a green fuel to replace petrol and therefore reduction of greenhouse gas emission
- Different methods of pretreatment evaluated to select the most effective method

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